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The Journal of LABORATORY and CLINICAL MEDICINE

VOL. 44 DECEMBER, 1954 NO. 6

The Official Publication of the Central Society for Clinical Research

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Vol. 44, No. 6, December, 1954. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription price: United States and its Possessions $12.00 per year; Canada $12.50; Foreign $13.50. Subscriptions to medical students, interns and residents, half-price. Entered as Second Class Matter October 8, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

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The Journal of Laboratory and Clinical Medicine

VOL. 44 DECEMBER, 1954 No. 6

CLINICAL AND EXPERIMENTAL

PENICILLIN PROPHYLAXIS OF EPIDEMIC STREPTOCOCCAL INFECTIONS

II. The Effects of Small and Large Doses of Oral Penicillin on Epidemic Streptococcal Infections and on Carriers of Group A Streptococci

JOHN R. SEAL, COMMANDER (MC) USN,* WILLIAM J. MOGABGAB, LIEUTENANT (MC) USNR, GEORGE J. FRIOU, LIEUTENANT (MC) USNR,** AND JAMES E. BANTA, LIEUTENANT, JUNIOR GRADE (MC) USN***

GREAT LAKES, ILL.

INTRODUCTION

THE successful use of oral penicillin for the prophylaxis of streptococcal infections and recurrent rheumatic fever in various civilian populations has been reported.[1-7] Military interest in use of penicillin prophylaxis during epidemic streptococcal infection stems from the magnitude of the problem in certain elements of the armed forces during mobilization and from the essential failure of methods other than chemoprophylaxis to control streptococcal epidemics.[8] The several investigations carried out in military populations during the past few years[9-12] have been more or less coordinated in an effort to obtain the basic information needed for the planning of economical and effective programs for the control of epidemic streptococcal infections. Studies among Navy recruits were begun in the spring of 1951[11] and have been summarized in

From the Naval Medical Research Unit No. 4, U. S. Naval Training Center, Great Lakes, Ill., and the Streptococcus Research Laboratory, United States Naval Training Center, Bainbridge, Md. Research Project NM 005 051.18, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

The opinions and assertions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or of the Naval Service at large. The usage of commercially available products in connection with the study cannot be construed as indicating endorsement or approval of such products or as preference for one product over other similar products which are or may become commercially available.

Received for publication, July 16, 1954.

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a preceding paper.[13] Briefly, it was found that daily oral doses of penicillin ranging from 50,000 to 125,000 units daily were about equally effective in preventing uninfected men from becoming infected. Larger doses, ranging between 375,000 and 500,000 units daily, were somewhat more effective than the small doses in preventing men from acquiring new strains of streptococci in their noses and throats and were much more effective in eradication of the organisms from men already infected. A majority of the studies had been made in relatively small groups of men.

The present study was a further investigation of the effects of two dosage regimens of oral penicillin during an epidemic of scarlet fever and other streptococcal infections. Entire regiments of Navy recruits were given courses of oral penicillin in a dosage of either 50,000 units or 500,000 units daily for two- or three-week periods. The effects of these courses of prophylaxis on admission rates for streptococcal infections, on carrier rates, on the distribution of specific types of streptococci in patients and in carriers, and on admission rates for rheumatic fever are dealt with in this report. The epidemiologic and clinical observations on streptococcal and other infections, as well as much of the materials and methods, and detailed bacteriologic results have been presented elsewhere.[13]

MATERIALS AND METHODS

Period of Study.—Most observations were made between January 6 and May 30; some extended from January 1 through June 25, 1953.

Populations.—Studies were conducted in the first, second, and fourth regiments of recruits and in the recruit preparatory training unit.[13] The regiments formed essentially independent units made up of companies of recruits in all stages of training. In the fourth regiment, all companies were regular Navy recruits undergoing eleven weeks of training. In the first and second regiments, about one-fourth of the companies were Naval reservists undergoing seven weeks of training.

Penicillin Prophylaxis.—Tablets containing either 50,000 or 250,000 units of buffered penicillin G were obtained from commercial sources. Arrangements were made with the Recruit Training Command to administer the tablets with sufficient supervision to ensure that all men eligible to receive prophylaxis received the prescribed dose. Bottles were prepared to contain the approximate number of tablets each company required each day and delivered to battalion officers who supervised the administration. These bottles were returned with unused tablets and a list of men in each company missing administration for any reason. Companies receiving a single daily dose of 50,000 units were given tablets at 5:30 A.M. Those given two 250,000 unit doses a day received tablets at 5:30 A.M. and at about 9:00 P.M. The schedules of administration and dosages employed were as follows:

REGIMENT	DAILY DOSE IN UNITS	INCLUSIVE DATES OF ADMINISTRATION	
		FIRST COURSE	SECOND COURSE
Fourth	500,000	2/ 8-2/21	4/ 5-4/25
Second	50,000	2/15-2/28	3/22-4/11
First	50,000	None	3/22-4/11

Prior to beginning prophylaxis, men were warned that they should not take the tablets if they had previously had a penicillin reaction. Questionnaires desiged to verify a previous reaction were completed by men applying for an excuse from prophylaxis. About 2 per cent of the regimental strengths were excused from prophylaxis on the basis of what seemed to be valid histories. A majority of these men were cultured and a group A streptococcus isolated in only five instances. Only one of the excused men was admitted with a streptococcal infection during any period of prophylaxis.

Admission Rates for Total Respiratory Infections.—A daily report prepared by the Medical Department listing the names of all admissions to the infirmary or Naval Hospital, the admission diagnoses, and the companies or other units to which the man belonged was available. Daily muster strengths of each company and the total available regimental strength were received from each recruit regiment. These reports included only men actually on duty and excluded men sick, on leave, or absent from their company for any other reason. From these reports average weekly strengths were calculated.

The record of each patient examined at the laboratory was compared with the daily medical department report and the admission diagnosis entered. Only those patients on whom the admitting physician and our examiner had agreed as having an acute respiratory infection were used in calculation of the total number of admissions each week. Where the patient had not been referred to the laboratory, the admitting physician's diagnosis was accepted and the case included in the weekly total. Only 18 per cent of patients diagnosed as having acute respiratory infections were not examined at the laboratory. A majority of these patients were ill and admitted outside of the hours of the three daily sick calls; many were diagnosed as having scarlet fever.

From the total weekly admissions for acute respiratory infections and the average strengths, weekly admission rates per 1,000 men in each regiment could be calculated.

Admission Rates for Streptococcal Infections.—Cases classified as having streptococcal infections were those with the following clinical, laboratory, and bacteriologic findings.[13] The categories are exclusive:

1. Scarlet fever.

2. Cases of acute respiratory infection with positive throat cultures for group A streptococci and:

(a) exudative tonsillitis or pharyngitis; (b) dysphagia and very tender anterior cervical nodes; (c) dysphagia or very tender anterior cervical nodes and a total leukocyte count of 12,000 or higher; (d) systemic symptoms and a total leukocyte count of 12,000 or higher; (e) fever of 100° F. or more and total leukocyte count of 12,000 or higher; and (f) a total leukocyte count of 16,000 or higher.

Scarlatinal rash, exudate in the pharynx, dysphagia, very tender cervical nodes, leukocytosis, and a positive throat culture for group A streptococci were the essential features of these criteria. The presence or absence of these findings either alone or in some combination determined the diagnosis. Complicated cases, those with immunization reactions or immunization within twenty-four hours, pneumonia, or a duration of symptoms of 120 hours or more were not classified as streptococcal infections unless they also had either scarlet fever or exudative tonsillitis or pharyngitis.

In weeks where not all patients had been examined, the percentage of examined patients classified as having streptococcal infections was determined. This percentage was then taken of the total number of admissions for acute respiratory infections and the resulting number recorded as the number of admissions for streptococcal infections during the week. Weekly admission rates for streptococcal infections were then calculated on the same basis as the total respiratory disease rates.

Admission Rates for Nonstreptococcal Infections.—Included here were cases of acute respiratory infection which had none of the findings which were considered to be of primary importance in the classification of infections as being of streptococcal etiology. The same exclusions were made and weekly admission rates calculated in the same way as for streptococcal infections.

Admission Rates for Patients With Various Individual Manifestations of Respiratory Infection.—The same methods were used to calculate admission rates for patients with the several clinical or laboratory findings which were considered of major importance in the recognition of streptococcal infections. In these calculations all patients were used without regard to classification. In the calculations of rates for patients with leukocytosis, however, patients with immunizations within twenty-four hours, primary vaccination reactions, pneumonitis, and pyogenic complications were excluded.

RESULTS

Effect on Admission Rates for All Acute Respiratory Infections and for Streptococcal Infections.—The weekly admission rates for all acute respiratory diseases and for patients meeting the criteria for classification as being streptococcal etiology from the three recruit regiments are shown in Figs. 1-3. Also shown in these figures are the weekly admission rates for patients exhibiting the several manifestations of respiratory tract infection which were important in the recognition of streptococcal infections and the admission rates for patients exhibiting none of these manifestations.

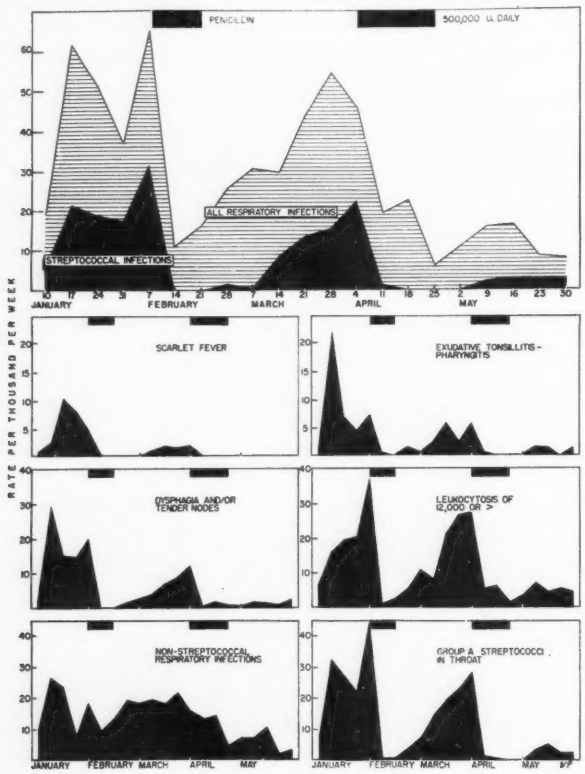

Fig. 1.—Admission rates for acute respiratory infections and for patients with various clinical and laboratory findings in relation to oral penicillin prophylaxis with 250,000 units twice daily, fourth regiment.

Beginning on February 3, all men in the fourth regiment received two tablets of sodium bicarbonate daily under the impression they were receiving penicillin. The medical officers at the regimental dispensary, the staff of recruit training, and most of our own personnel were also led to believe that penicillin prophylaxis was started on that date. At the evening dose on February 7, the 250,000 unit tablet of penicillin was substituted for the placebo which it closely resembled in size and shape. No one other than our own personnel was informed of the change.

The lack of effect of the placebo is obvious, there being a sharp rise in admission rates while it was being given. On February 8, when only two doses of penicillin had been administered, only three cases which could be considered of streptococcal etiology were admitted. Two were scarlet fever and were not referred to the laboratory for examination but confirmed at the hospital. The other man had symptoms of more than seventy-two hours' duration; fever, exudative tonsillitis, a leukocytosis, and negative throat culture. He sought treatment only at his parents' insistence when they visited him on that day. Symptoms had abated during the twenty-four hours prior to admission. No other patients meeting the criteria for classification as streptococcal infections were admitted during the two weeks of prophylaxis.

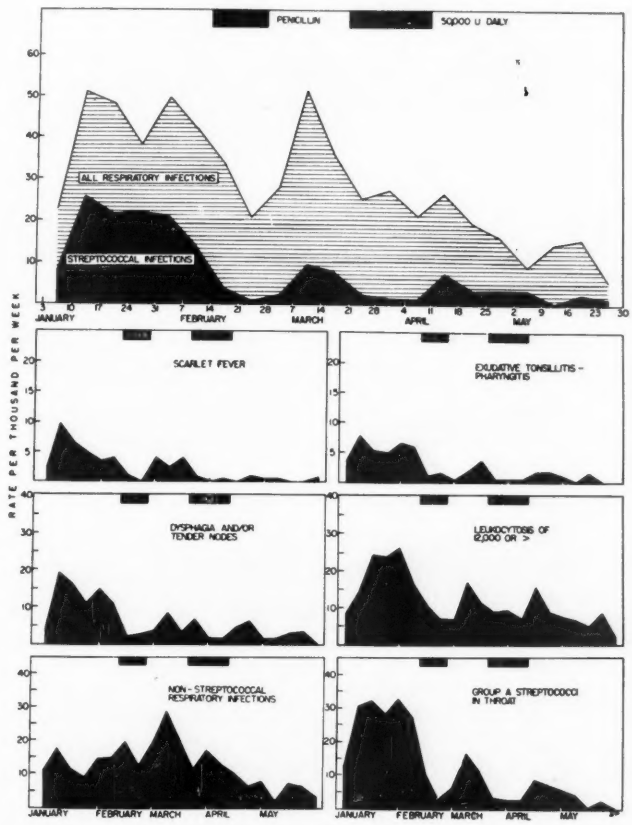

Fig. 2.—Admission rates for acute respiratory infections and for patients with various clinical and laboratory findings in relation to oral penicillin prophylaxis with 50,000 units once daily, second regiment.

There was also an abrupt and profound fall in the admission rate for all respiratory infections from 64.9 to 11.0 per 1,000 per week. This fall was in excess of the fall in admission rate for cases classified as streptococcal infections, which was from 31.3 to nearly zero per 1,000 per week. This indicates that the majority of admissions were in some way related to epidemic streptococcal infections in this regiment during the week preceding prophylaxis and that other

diseases were not epidemic in the population at the time. When 500,000 units daily was again given to this regiment six weeks after the first course, at which time the admission rate for all respiratory infections was 45.5, the fall was to 19.6, a difference of 25.9. Streptococcal admissions fell from 22.3 to 1.2, a difference of 21.1. These differences more nearly coincide.

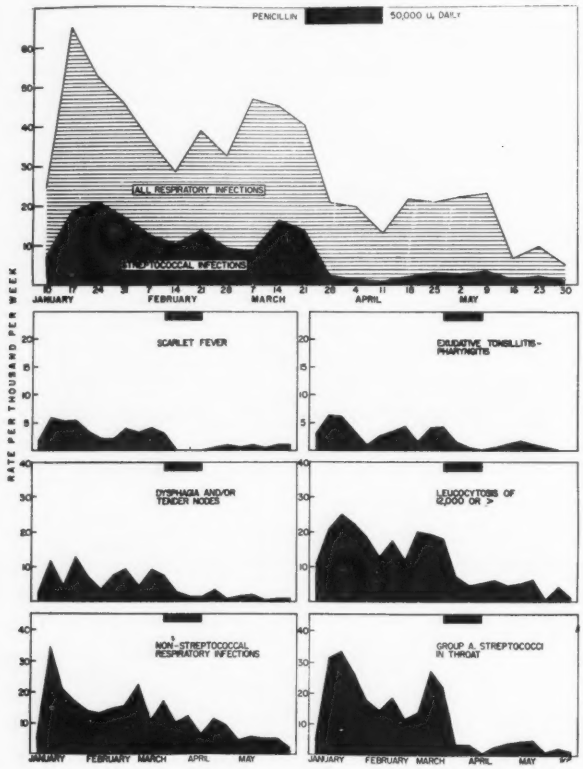

Fig. 3.—Admission rates for acute respiratory infections and for patients with various clinical and laboratory findings in relation to oral penicillin prophylaxis with 50,000 units once daily, first regiment.

In view of the obvious lack of effect of placebos on admission rates in the fourth regiment, they were not used in the second regiment. This regiment was started on a daily dose of 50,000 units on the night of February 14. Introduction of penicillin brought about a gradual decline in admissions for cases classifiable as streptococcal infections over the two-week period of administration. In contrast to the fourth regiment, where admission rates gradually returned to preprophylaxis levels, the admission rates for streptococcal infections quickly rose in the second regimént when prophylaxis was stopped. This behavior of rates in the postprophylaxis period was repeated following the second course of prophylaxis in the second and fourth regiments. In general, the decline in admission rates for all respiratory infections in the second regiment during the two courses of prophylaxis paralleled and equaled the decline in streptococcal infections.

Admission rates for streptococcal infections from the first regiment, which was not given prophylaxis at the time of the first course in the other two regiments, were maintained above 10 per 1,000 per week for nearly all weeks until prophylaxis was given.

More illustrative of the actual trend of events during prophylaxis than were admission rates was the daily number of admissions for streptococcal infections from the three regiments.

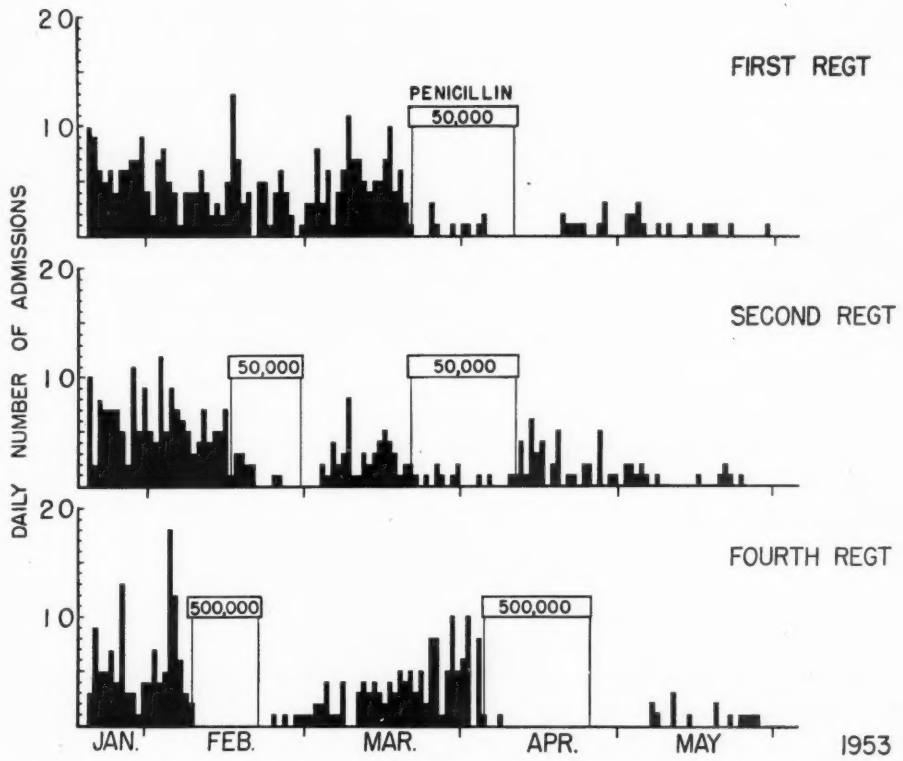

Fig. 4.—Daily admissions for patients with streptococcal infections from three regiments of Navy recruits before, during, and after oral penicillin prophylaxis.

In Fig. 4 the abrupt cessation of admissions in the fourth regiment and the slow return of admissions to their former level are shown. Contrasting is the continued admission of streptococcal infections during administration of 50,000 unit doses of penicillin in the first and second regiments and the rapid return after prophylaxis was discontinued.

Effect of Prophylaxis on Streptococcal Infections Caused by Type 19 Streptococcus.—Type 19 streptococcus was epidemic in all three regiments and caused nearly all cases of scarlet fever.[13, 14] As one objective of the study had been to compare the effects of the 50,000 and 500,000 unit doses of oral penicillin on the elimination of the strains which were prevalent at the time of initiation of prophylaxis, the data were analyzed to show the number of

admissions classified as streptococcal infections which had been caused by Type 19 streptococci. The results are shown in Table I.

In the twenty-one-day period following the use of 500,000 units of oral penicillin in the fourth regiment, only seven patients with streptococcal infection caused by this type were admitted. This is only one-fifth as many as were admitted from the second regiment following the 50,000 unit dose. These data illustrate the rapid return of streptococcal infections following the small dose and the slower return following the larger dose. After the second course of prophylaxis, infections caused by Type 19 streptococcus were not admitted from the fourth regiment in a twenty-eight-day period. Again the rapid return in the two regiments given 50,000 units is seen, although not to the preprophylaxis levels.

TABLE I. NUMBER OF NAVY RECRUITS ADMITTED WITH STREPTOCOCCAL INFECTIONS CAUSED BY TYPE 19 STREPTOCOCCUS FROM REGIMENTS RECEIVING ORAL PENICILLIN PROPHYLAXIS WITH 50,000 UNITS ONCE DAILY OR 250,000 UNITS TWICE DAILY IN TWO- OR THREE-WEEK COURSES

COURSE OF PROPHYLAXIS		FIRST				SECOND			
RELATION TO COURSE		BEFORE	DURING	AFTER		BEFORE	DURING	AFTER	
DAYS OF OBSERVATION		14	14	14	21	21	21	21	28
First Regiment	Number admitted	32	40*	41	56†	56†	3	7	12
	Daily dose penicillin	None	None	None		None	50,000 u.		None
Second Regiment	Number admitted	35	5	20	35†	35†	5	12	19
	Daily dose penicillin	None	50,000 u.	None		None	50,000 u.		None
Fourth Regiment	Number admitted	47	0	3	7	38	0	0	0
	Daily dose penicillin	None	500,000 u.	None		None	500,000 u.		None

*First regiment not given first course of prophylaxis.
†Total elapsed time between first and second courses, 21 days.

Effect of Prophylaxis on Nonstreptococcal Infections.—Admission rates for nonstreptococcal infections are also shown in Figs. 1-3. There were no definite changes of these rates in relation to the courses of penicillin prophylaxis.

The discrepancy in these figures between admission rates for all respiratory infections and for streptococcal infections can only be partially accounted for by the admission rates for patients having illness considered to be definitely of nonstreptococcal etiology. There remains a residue of miscellaneous infections excluded from being definitely classified as of either streptococcal or nonstreptococcal etiology. Some of these were patients with negative cultures which exhibited one or more of the clinical features that had been considered of major importance in the recognition of streptococcal infections. Others were patients excluded for reasons previously stated.

During the twenty-five weeks of study, fifty-nine radiologically confirmed pneumonias were admitted from the first regiment, seventy-nine from the second, and forty-eight from the fourth. A majority received a diagnosis of primary atypical pneumonia at the hospital. When analyzed in relation to the courses of prophylaxis, there was no evident effect of either dosage of penicillin. The small number of cases admitted in any short interval, however, and the short periods of prophylaxis prevented any critical appraisal.

Rubella occurred in all three regiments during the entire period of study. Peak rates were attained in March and April when the incidence varied between five and nine per one thousand per week. Prophylaxis had no effect on admission rates for this infection.[13]

Effect on Several Clinical and Laboratory Features of Respiratory Infection Attributed Importance in Recognition of Streptococcal Infection.—An analysis of bacteriologic findings in patients admitted during prophylaxis with the 50,000 unit dose had suggested that a part of the effect of this dose may have been to increase the error of throat culture by reducing the streptococcal population of the throat and that the reduction in numbers of cases of streptococcal infection may have been to some extent an artifact because of the dependence of diagnostic criteria on positive cultures.[13] That this was not the only effect is shown in Figs. 1-3.

A pronounced effect of both the small and larger doses of penicillin is seen on the admission rates for scarlet fever. The effect is most marked and persistent in the fourth regiment with the daily dose of 500,000 units. The effect of the larger dose on exudative tonsillitis or pharyngitis was also to terminate completely admissions for patients with this finding, whereas a few cases continued to occur in men receiving the small dose.

Changes in the admission rates for patients exhibiting other clinical and laboratory findings accorded importance in the diagnosis of streptococcal infection followed the same pattern as had the rates for scarlet fever and exudative tonsillitis or pharyngitis. As the patients represent the proportion of total admissions exhibiting these manifestations, the marked changes in the clinical picture of respiratory infections which occurred during the prophylactic periods are evident.

Effects on Admission Rates for Streptococcal Infections Caused by All Prevalent Strains of Group A Streptococci.—It was of interest to follow the behavior of admission rates for streptococcal infections caused by the several serologic types of streptococci which were encountered with varying degrees of frequency during the study. The numbers of Type 19 infections were summarized in Table I and the admission rates are shown in Table II.

In Table II are shown the weekly admission rates for streptococcal infections caused by Types 19, 3, 1, and 12 streptococci. The combined rates for types other than these four which were prevalent are also included under the heading of "other."

Types other than 19, 3, 1, and 12 were encountered only sporadically. No more than six isolations of any of the eight other types identified were made from a regiment during the entire twenty-five weeks of study.

In the fourth regiment, Types 19, 1, and 12 were prevalent prior to the first course of prophylaxis. Subsequently, more than two weeks elapsed before another Type 1 and three weeks before another Type 12 infection was admitted. Type 19 reappeared during the first week after prophylaxis and slowly increased but did not regain its former prevalence within six weeks. Two Type 3 infections were admitted in the week prior to prophylaxis. Following prophylaxis, this type became as prevalent as Type 19. Type 3 first appeared following

TABLE II. AVERAGE WEEKLY STRENGTH AND ADMISSION RATES FOR STREPTOCOCCAL INFECTIONS CAUSED BY SEROLOGIC TYPES OF GROUP A STREPTOCOCCI IN THREE REGIMENTS OF NAVY RECRUITS IN RELATION TO EFFECTS OF ORAL PENICILLIN PROPHYLAXIS FOR TWO- OR THREE-WEEK PERIODS BETWEEN JANUARY AND JUNE, 1953

WEEK ENDING	FIRST REGIMENT AVERAGE WEEKLY STRENGTH	FIRST REGIMENT ADM/1000/WK. WITH TOTAL GR. A	TYPES 19	3	1	12	OTH.	SECOND REGIMENT AVERAGE WEEKLY STRENGTH	SECOND REGIMENT ADM/1000/WK. WITH TOTAL GR. A	TYPES 19	3	1	12	OTH.	FOURTH REGIMENT AVERAGE WEEKLY STRENGTH	FOURTH REGIMENT ADM/1000/WK. WITH TOTAL GR. A	TYPES 19	3	1	12	OTH.
Jan. 10	2671	4.8	4.4		0.4			2692	5.2	5.2					2534	3.7	3.3		0.4		
17	2816	13.9	13.4		0.5			2772	17.5	17.5					2506	16.1	16.1				
24	2686	17.3	13.1		2.8	1.4		2538	18.9	16.4		1.9		0.6	2284	16.1	14.7		1.4		
31	2658	15.1	13.0		1.7	0.4		2416	19.2	14.8		3.3		1.1	2214	15.7	12.8		2.3	0.6	
Feb. 7	2522	11.0	6.9		1.8	0.5	1.8	2310	19.3	8.4	1.9	3.3	3.7	2 3	2113	29.5	14.5	0.6	6.6	6.0	1.8
14	2511	9.0	6.2		0.8	0.8	1.2	2092	13.8	7.9		3.9	1.0	1.0	**1972**	**0.1***					
21	2568	11.9	9.9		0.8	0.4	0.8	**2061**	**3.5**	**1.5**		**0.5**	**1.0**	**0.5**	**1930**	**0.0**					
28	2621	7.2	6.8		0.4			**2268**	**1.0**	**1.0**					1971	1.7	1.7				
																		3.3			0.6
Mar. 7	2611	8.0	6.0		0.8	0.4	0.8	2510	2.4	2.0			0.4		1999	3.9	2.2	2.8	1.7		0.6
14	2618	14.3	9.9	0.8	2.0	1.2	0.4	2492	8.9	7.1	0.9	0.9			2012	7.3	5.9	2.3	1.8	1.2	0.6
21	2653	12.4	5.6	3.4	3.4			2536	7.1	5.9	0.8		0.4		2102	11.8	6.8	6.8	0.5	0.5	
28	**2787**	**1.9**	**0.4**	**0.4**	**1.1**			**2599**	**2.4**	**1.2**	**0.4**		**0.4**	**0.4**	2248	14.6					
																	8.3	9.7	3 9		
Apr. 4	**2806**	**1.5**	0.7		**0.4**	0.4		**2446**	**1.0**		**0.5**		**0.5**		2231	21.9		1.2			
11	**2607**	**1.0**			**0.5**		**0.5**	**2366**	**0.9**	**0.9**					**2165**	**1.2**					
18	2397	1.6	0.8		0.4	0.4		2400	5.9	1.3	2.9	1.7			**2113**	**0.0**					
25	2280	2.8	1.4	1.4				2398	3.7	1.8	1.4	0.5			**2007**	**0.0**					
May 2	2190	2.7	2.7					2327	3.7	1.4	2.3				1873	0.0					
9	2046	2.9	2.4				0.5	2232	3.5	2.5	0.5	0.5			1687	0.8		0.8			
16	1981	1.2	0.6	0.6				1995	0.5		0.5				1606	2.9		**2.9**			
23	2103	2.1	2.1					1711	2.7	0.6	1.9				1506	1.9		**1.9**			
30	2230	1.0	1.0					1389	0.0						1381	3.0	1.5	1.5			

Bold-faced type indicates weeks during which 50,000 units penicillin daily given to first and second regiments, 500,000 units daily to fourth regiment.

*Two cases scarlet fever not examined and presumably due to Type 19 streptococcus.

prophylaxis in a new company which had entered the regiment during the second week of prophylaxis and received penicillin for only a few days. Six of nine admissions for streptococcal infections during the second week following prophylaxis were caused by this one type and they were all from this company. Unfortunately, men making up this company were not cultured upon arrival on the station nor was it determined whether men who had been on the station for some time and were carriers of this type were included in the company prior to the outbreak. The second course of prophylaxis in the fourth regiment virtually stopped all admissions for streptococcal infections except those caused by Type 3; this type again appeared the second week after the course was completed.

It is difficult to assess the effect of the 50,000 unit dose on any strains except Type 19 in the first and second regiments because of the short interval between prophylaxis courses in the second regiment and the late date of the first course in the first regiment. In the second regiment, the effect on Type 19 was short-lived as were any possible effects on Type 3. Type 3 did not appear in the first regiment until the week ending March 14. This was of interest inasmuch as the only difference between the three regiments in potential sources of introduction was that the second and fourth regiments shared the same mess hall at the time this strain first appeared.

Effect of Prophylaxis in Recruit Preparatory Training Unit.—This small population was the best segregated group of male recruits on the station. As described elsewhere,[13] the men had little daily contact with anyone other than their instructors. Exceptions were men attending sick calls at the fourth regimental dispensary, who were hospitalized, needed dental treatment, or were disciplinary problems. Thus, the isolation of this unit was not complete and opportunities existed, particularly in the case of men returning from hospitalization, for introduction of strains of streptococci from elsewhere on the base.

In the five-week period between January 6 and February 7, there were fifty-one men hospitalized for respiratory infection from this population which had an average weekly strength of 233 men for the period. Influenza was mainly responsible for this high morbidity.[13] Thirty-five of the fifty-one men were examined at the laboratory. Ten had streptococcal infection (29 per cent). A total of fourteen had a positive throat culture for group A streptococci. At a throat culture survey on February 2, sixty-five of the 244 men in the unit were cultured. Selection for culturing was made by picking every fourth name from an alphabetized list of names of men in the unit. Fifteen (23 per cent) had positive throat cultures for Group A streptococci. Type 1 was isolated from seven, Type 5 from one, Type 19 from two, and untypable strains from five.

Prophylaxis with 250,000 units of oral penicillin twice daily was given from February 7 through February 21. Admissions following the influenza epidemic and this course of prophylaxis were spotty, a total of only thirty-four additional men being examined between February 8 and June 25.

TABLE III. PER CENT OF THROAT CULTURES TAKEN ON ABOUT ONE-QUARTER OF EACH OF THREE NAVY RECRUIT REGIMENTS POSITIVE FOR VARIOUS SEROLOGIC GROUPS AND TYPES OF BETA HEMOLYTIC STREPTO COCCI IN RELATION TO TWO- AND THREE-WEEK PERIODS OF ORAL PENICILLIN PROPHYLAXIS

REGIMENT	CULTURE SURVEY DATE	NUMBER MEN CULTURED	PER CENT CULTURES POSITIVE FOR BETA HEMOLYTIC STREPTOCOCCI								
			GROUP A								NOT GROUP A
				SEROLOGIC TYPES							
			TOTAL	19	1	3	5	12	OTHER*	NCT†	
First	Feb. 13	557	10.6	4.1	2.9			0.2	0.4	3.1	4.3
	Mar. 17	612	17.7	9.5	2.8	1.5	0.2	1.1	0.5	2.1	2.6
	Apr. 6	**651**	**6.5**	**2.6**	**1.1**	**0.8**		**0.8**	**0.2**	**1.1**	**0.8**
	13	595	10.4	3.0	2.5	0.8	0.2	1.2	0.3	2.4	1.5
	20	570	9.1	2.8	2.3	0.5	0.5	0.7		2.3	3.2
	27	586	7.5	0.7	1.4	1.5	0.5	0.9		2.6	2.4
	May 12	529	2.8	0.9	0.4	0.6		0.2		0.8	1.5
Second	Feb. 10	559	21.1	6.4	5.0	0.5	0.4	5.7		3.0	8.4
	20	**581**	**12.6**	**5.7**	**2.4**	**0.5**	**0.3**	**1.0**	**3.4**	**2.2**	**4.6**
	Mar. 7	588	8.8	2.9	1.4	2.2	0.5	0.3	1.7	1.5	4.8
	19	671	10.3	4.2	0.5	1.6	0.9	0.7		2.4	3.1
	Apr. 1	**597**	**7.4**	**2.9**	**0.3**	**1.5**	**0.3**	**1.0**	**1.7**	**1.8**	**1.5**
	9	609	8.1	3.3	1.0	1.6	0.5	0.2		1.5	1.8
	15	542	9.4	2.6	0.7	2.2	0.4	0.6	1.9	2.8	1.3
	22	666	8.3	2.6	0.8	2.4	0.2	0.5	1.5	1.8	2.1
	29	583	8.7	2.1	1.0	3.9	0.3		1.7	1.2	2.2
	May 13	531	5.5	1.1	0.4	1.7	0.2	0.2		1.9	2.1
Fourth	Feb. 4	567	32.3	11.1	9.5	0.7	0.2	6.2	5.3	4.1	7.6
	20	**416**	**1.2**							**0.5**	**0.2**
	Mar. 2	478	1.3	0.2	0.2	0.8					2.5
	12	476	6.7	1.7	0.4	3.2	0.2	0.8		0.4	3.0
	Apr. 3	538	19.5	3.7	3.4	8.6	0.4	1.3	1.9	2.0	**0.2**
	17	**471**	**0.6**		**0.2**	**0.2**	**0.4**			**0.2**	**0.4**
	24	**521**	**1.2**	**0.2**	**0.2**	**0.8**					0.6
	May 1	472	3.2	0.4	0.6	1.3			4.2	0.6	0.6
	8	465	5.4	0.6	0.2	0.9				**3.7**	1.9
	20	367	4.9	0.5	0.5	2.7				1.1	**0.3**
	June 5	310	2.6	1.0	0.3	1.3					

Bold-faced type indicates cultures taken while on prophylaxis. First regiment 50,000 units daily Mar. 25 to Apr. 11. Second regiment 50,000 units daily Feb. 24 to Feb. 28 and Mar. 25 to Apr. 11. Fourth regiment 500,000 units daily Feb. 8 to Feb. 21 and Apr. 5 to Apr. 26.

*Types 2, 6, 7, 11, 14, 18, 28.

†Did not type in available antisera.

One man was admitted on February 27 with an exudative pharyngitis, leukocytosis, pure culture of streptococcus Type 1, and symptoms of twenty-seven hours' duration, just three days after his return from the hospital. He had not received prophylaxis or been cultured at the preprophylaxis survey because of his previous hospitalization. The next admission for a streptococcal infection from this unit was on March 17 and was of a man added to the unit following prophylaxis. The next admission for a streptococcal infection was in June. In all, only five positive throat cultures were obtained in the thirty-four men examined. Nineteen of the remaining twenty-nine men with negative throat cultures had common coldlike illnesses, most of which were afebrile.

Throat culture surveys were also made on February 17, March 2, and March 26. Of the sixty men cultured during prophylaxis and the fifty-nine men cultured ten days after it was concluded, none were positive. Four of sixty-one men cultured at the last survey were positive, one for Type 1, one for Type 19, and two for untypable strains. The Type 19 was a weakly positive culture with glossy colonies in a man who had no illness and whose previous cultures had been negative. The Type 1 was a strongly positive culture with mucoid colonies in a man with respiratory symptoms who had been added to the unit since prophylaxis. Both of the untypable strains were isolated from weakly positive cultures from new men in the unit, one of whom had no symptoms, and the other had had a sore throat three weeks previously.

Thus, in this isolated unit, prophylaxis with 250,000 units of oral penicillin twice daily seemed able to eradicate streptococcal infections almost permanently.

Effect on Carriers of Group A Streptococci.—Any man on duty with his company at the time a positive culture was obtained was termed a "carrier." Among men with positive cultures some probably had or were convalescent from clinical streptococcal infections for which they may not have sought treatment; some had probably had subclinical infections and were still carrying the organism; some were chronic carriers; and others were probably the sporadic positives which have been found in groups of recruits serially cultured during epidemics.[8] It was not feasible to attempt differentiation.

Throat cultures were obtained from approximately one-quarter of the men in each regiment at intervals. Men were selected for the initial culture by picking every fourth name from an alphabetized roster of the names of men in each company in the regiment. New companies entering training after the initial culture survey were similarly treated. At subsequent surveys the same men returned plus one-fourth the number of any new men added to each company since the last survey. The throat culture was obtained by a medical officer under direct vision. All strains of streptococci isolated were serologically identified and tested for penicillin resistance.

The summarized results in all three regiments are shown in Table III. Also shown in this table are the dates of the culture surveys and the relationship to penicillin prophylaxis. The distribution of serologic types of streptococci was essentially the same as that found in patients with streptococcal infections and in other populations on the station.

The result of prophylaxis with the 500,000 unit daily dose in the fourth regiment was dramatic. Type 19, the most prevalent type before prophylaxis, was markedly reduced by the first course and never regained its prevalence in the six-week interval between courses. With the exception of Type 3 streptococci, the effect on the other prevalent types was equally marked. Type 12 streptococci were virtually eradicated. Type 3 streptococci were not prevalent before prophylaxis. In the interval between the first and second courses of prophylaxis this strain increased in prevalence and at the time the second course was begun was the most prevalent type. It persisted through the second course of prophylaxis.

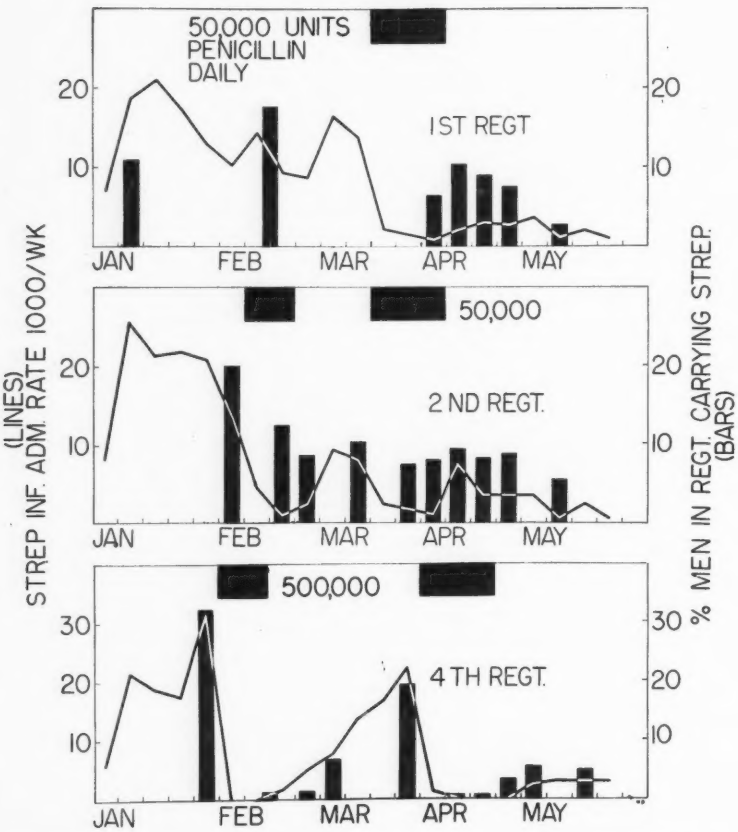

Fig. 5.—Effect of oral penicillin prophylaxis in three regiments of Navy recruits on admission rates for streptococcal infections and on carrier rates.

The 50,000 unit dose of penicillin appeared to reduce carrier rates to some degree but had no effect comparable to that following the larger dose. This is illustrated in Fig. 5 where the carrier rates and admission rates for streptococcal infections are compared. The 50,000 unit dose had a greater effect on admission rates than on carrier rates. The 500,000 unit dose dramatically reduced both rates, exhibiting both prophylactic and therapeutic actions.

The data were further examined in an effort to obtain information on possible sources of the recurrent high incidence of admissions for streptococcal infections and of carriers of these organisms in the fourth regiment.

At the survey on February 20, just before discontinuance of the first course of prophylaxis, two men of those cultured had positive cultures. Both were in companies in the last weeks of training and the strains did not type in available antisera. All other cultures were negative including those on the appreciable percentage of men who entered the companies of this regiment from the hospital during prophylaxis. Eight new companies had entered training during prophylaxis and men from these companies all had negative cultures.

At the next survey on March 2, ten days after penicillin had been discontinued, a total of fourteen men were found to have positive cultures for Group A streptococci. One had a Type 19, four a Type 3 streptococcus, and the rest of the strains did not type in available antisera. One man with a strongly positive culture for a Type 3 streptococcus had entered a company following his discharge from the hospital on February 18 and could have received at most only two or three days of prophylaxis. Another man from this same company yielded one of the other three strains for Type 3. The fourteen men were from seven different companies in all stages of training. Seventeen other companies in which no positive cultures had been found at the previous survey were again present and all men cultured from these companies had negative cultures. The men from two new companies which had entered the regiment since prophylaxis also had negative cultures.

At the survey on March 12, just ten days later, there were twenty men with positive cultures who had negative cultures at the previous survey. These twenty men were distributed among thirteen companies, again in all stages of training. Two men who had acquired positive cultures for a Type 1 streptococcus were in one of the oldest companies and this type was not found in other companies. Nine men from five companies had acquired Type 3 streptococci. Four of the nine men with positive cultures for this type were from one of the older companies. Five men, all from different companies, had acquired Type 19 streptococci. Two men with positive cultures for Type 3, and one man with a Type 19 streptococcus were identified as having entered companies following discharge from the hospital since the previous survey. Each of these companies had contributed other men with new acquisitions to the previously mentioned total. Men positive at the previous survey also had positive cultures at this one, and other men not cultured at that survey who may or may not have been added to the companies from unidentified sources during the interval between surveys were found with positive cultures for Types 3, 19, 12, and 5 streptococci. All men cultured from seven of the seventeen companies with negative cultures at the last survey were again negative. Five new companies had joined the regiment during the preceding ten days. In four, all men cultured had negative cultures. In the other there was one man with a weakly positive culture for a Type 5 streptococcus. This type was not found in other men of the company at subsequent surveys.

The next survey was not made until April 3, just before reinstitution of prophylaxis. At this time there were thirty-eight men who could be identified as having acquired streptococci since the last survey. These were from eighteen companies in different stages of training. Types 3, 19, 1, 12, and 5 had been acquired in that order of prevalence. Five men carrying Type 1 or 19 who had been recently discharged from the hospital were found in different companies. In one other company a man with a negative culture at the last survey had developed a sore throat, entered and been discharged from the hospital following penicillin treatment, and now had a positive culture for a Type 1 streptococcus. Twelve new companies had entered since the last survey. One hundred forty-seven men from these companies were cultured and eighteen had positive cultures. The types found included Types 1, 3, 5, 12, 14, and 19. One of the two youngest companies had no men with positive cultures, the other had one man with a Type 3. Both had been in training for nearly one week. Men recently discharged from the hospital were cultured in four of the twelve companies but all had negative cultures. In the newest company was a man who had been discharged from the hospital on March 19.

These observations would probably have been more conclusive had we been able to culture all men in every company and obtain more exact information as to which originally arrived from civilian life and which were added to the company from various sources during training. Despite this deficiency, these data strongly suggest that the source of the recurrence of streptococcal infections in the fourth regiment was on the station and not within new recruits entering training from civilian life. Additional evidence to this effect was obtained from 285 recruits cultured at the receiving unit between February 25 and February 27. These men had been on the station between forty and 120 hours. Group A streptococci were isolated from five men, one being a Type 12, one a Type 19, and the other three not typing in available antisera. All had less than ten colonies described as being glossy on the direct streak plate. This was in contrast to the frequent finding of matt or mucoid colonies and strongly positive cultures in the company surveys. It is possible that even those with positive cultures had acquired them since being on the station and it was not feasible to culture recruits on arrival from civilian life.

Observations on Streptococci in Populations Other Than Recruits in Training.—Streptococcal infections were frequently encountered in men from other populations during the study. Because observations in these other populations have a bearing on the potential source of Type 3 infections among recruits, they are included here. The populations have been described previously.[13] A total of eighty-two men with acute respiratory infections were admitted while in activities from which men may be added to the recruit regiments. Ten were from the receiving unit and only one man had a positive culture for an untypable strain. Forty-three were in the reassignment unit en route from hospital to regiments, sixteen (37 per cent) had positive cultures, most of which were Type 39. One man with a Type 3 streptococcal infection was admitted from the reassignment unit on January 30 just prior to the first discovery of this type in

the recruit regiments. Of twenty-nine men admitted while in the station brig or under psychiatric observation, thirteen were positive for Type 19, and two positive for Type 3.

The first appearance of Type 3 among patients admitted for respiratory infections was among service school students, being recovered from men admitted on January 24, 28, and 31. This type first appeared in patients admitted from the administrative command on February 5 about the time of its first isolation from recruits in training.

Among the admissions were also men from two-week reserve companies, the Naval Academy Preparatory School, the outgoing unit, and instructors from recruit training. Streptococcal infections occurred in all, and Type 19 was by far the most prevalent type from all groups except the Naval Academy Preparatory School.

A majority of the admissions from these other populations occurred during the influenza epidemic. Type 3 streptococci were not isolated from any unit prior to January 24. Following its first appearance in the Service Schools on that date, it spread rapidly among other populations and in March was identified in all populations. It is probable that the influenza epidemic in January with hospitalization of men from all populations on large open wards at the hospital had a lot to do with this rapid, widespread dissemination, as men hospitalized for influenza during the epidemic had just returned to their populations when the type first made its appearance in them. The finding of a streptococcal infection caused by this type in a recruit en route from hospital to a new company offers confirmatory evidence in this direction.

An additional 1,283 recruits in the receiving unit were cultured in surveys between May 5 and June 12. Thirty-seven (1.4 per cent) had positive cultures for Group A streptococci of which three were Type 3, two Type 5, seven Type 12, four Type 19, and twenty-one untypable. Information was not obtained about the length of their stay on the station prior to culture. All positive cultures were recorded as having less than ten colonies per plate.

Effect of Two Prophylactic Regimens on Carriers Followed Serially.—Since the over-all carrier rates do not differentiate between the effects of the antibiotics

TABLE IV. RESULTS OF THROAT CULTURES FOR GROUP A STREPTOCOCCI IN RECRUITS CULTURED BOTH BEFORE AND AFTER PERIODS OF PENICILLIN PROPHYLAXIS

COURSE OF PROPHYLAXIS	REGIMENT GIVEN PROPHYLAXIS DURING COURSE	DAILY PENICILLIN DOSAGE IN UNITS	TOTAL MEN CULTURED BOTH BEFORE AND AFTER PROPHYLAXIS	NUMBER WITH THROAT CULTURES FOR GROUP A STREPTOCOCCI			
				NEGATIVE BEFORE PENICILLIN		POSITIVE BEFORE PENICILLIN	
				REMAINED NEGATIVE	BECAME POSITIVE	REMAINED POSITIVE	BECAME NEGATIVE
First	2	50,000	195	142	17	3	33
	4	500,000	185	122	0	1	62
	1	50,000	183	144	9	17	13
Second	2	50,000	282	221	28	18	15
	4	500,000	214	168	1	3	42
Total	1 & 2	50,000	660	507	54	38	61
	4	500,000	399	290	1	4	104

in preventing new acquisitions of streptococci and in eradication of streptococci from men already infected, the data were examined in this regard. Between surveys, infected men were leaving training and uninfected men being added constantly to the population, so the analysis was confined to those men who had been cultured both before and immediately after each of the several courses of penicillin. Because of the rapid turnover and the length of the prophylactic regimens, a relatively small proportion of the total men cultured could be thus compared.

Of the 561 men who received 50,000 units of penicillin orally for two or three weeks and who had a negative culture at the preprophylaxis survey, 10 per cent had a positive culture at the postprophylaxis surveys conducted two to eight days after the end of the courses. Thirty-eight per cent of the ninety-nine men with positive cultures before prophylaxis still had a positive culture after two or three weeks of receiving 50,000 units of penicillin daily.

Only one of the 291 men with negative cultures before prophylaxis and who received 500,000 units of penicillin daily for two or three weeks had a positive culture after the end of prophylaxis. Postprophylaxis surveys were conducted five to ten days after the last dose. Four per cent of 108 men with positive cultures before prophylaxis remained positive. These results are in essential agreement with previous findings when doses in this order were employed.[12]

Effect of Prophylaxis on Rheumatic Fever.—During the study there were fifty-six cases of rheumatic fever admitted from the three recruit regiments. The diagnosis had been established by clinical and electrocardiographic study on the wards of the hospital. Migratory arthritis was the major manifestation in forty-eight of the fifty-six. Nine (16 per cent) had experienced one or more attacks of rheumatic fever previously. This was significantly different from the incidence of a past history of rheumatic fever in all patients with all respiratory diseases examined during this study where the incidence was 1.8 per cent. Only twenty-one of the fifty-six cases had been admitted or treated for the respiratory infection which immediately preceded the onset. Eighteen of the twenty-one had received penicillin and three chlortetracycline at the time of their respiratory infection. A Type 19 streptococcus had been isolated from seven of eleven patients examined at the laboratory for the streptococcal infection preceding the onset. Two had Type 3 streptococcal infections. One other had fever and leukocytosis with symptoms of an acute bronchitis and a negative culture. A type 19 streptococcus was isolated from seven of eleven other patients who were cultured at the laboratory at the time of admission for rheumatic fever. Cultures in the other four were negative but all had received antibiotic therapy recently.

Penicillin prophylaxis affected the incidence of rheumatic fever in all three regiments. In the first regiment there were fourteen cases of rheumatic fever in the fifteen weeks between January 1 and the end of prophylaxis. Only one case occurred between this time and June 20, an interval of eleven weeks.

In the second regiment, twenty-three cases occurred in a nine-week period before the end of the first course of prophylaxis. Only four cases occurred

during the six-week interval between the end of the first and second courses of penicillin and only one case following the end of the second course.

The results in the fourth regiment were more dramatic. Thirteen cases occurred in the eight weeks before the end of the first course of prophylaxis. None were admitted in the following eighteen weeks of observation, despite the recurrence of epidemic streptococcal infections in the population necessitating a second course of prophylaxis.

Acute Glomerulonephritis.—In view of the frequency with which this disorder occurred in association with Type 12 streptococcal infection at this station a year previously,[15] and the continued presence of this type of streptococcus in the recruit population, a search was made for cases on the hospital wards and

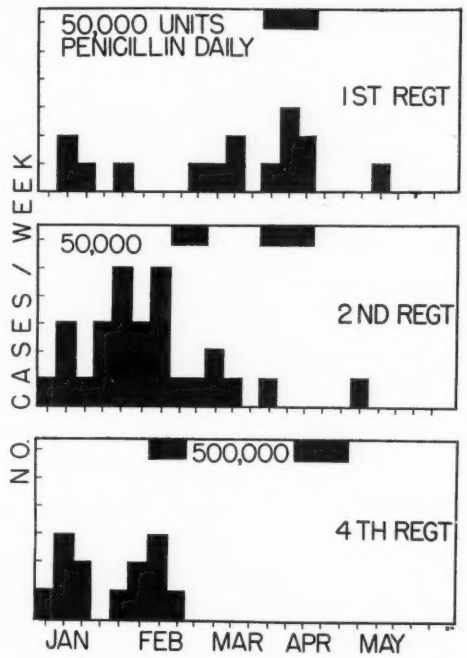

Fig. 6.—Week of onset of rheumatic fever in patients admitted from three regiments of Navy recruits before, during, and after oral penicillin prophylaxis.

in the records. A number of patients had been admitted with diagnoses of albuminuria, hematuria, or suspected nephritis. Six patients, all admitted in January or early February, had been diagnosed as acute glomerulonephritis by the ward physician on the basis of urinary abnormalities, but none of these had been found to have hypertension, edema, nitrogen retention, or any abnormalities other than the transitory finding of casts and red blood cells in the urine. None of the patients examined for Type 12 streptococcal infections developed any renal complications which were detected.

Hypersensitivity Reactions to Penicillin.—The cooperation from the recruits and from the staff of the Recruit Training Command in administration of the

prophylaxis was excellent. Men unavoidably missed some doses but on the whole there were probably few men in the three regiments who did not take a majority of the doses during each course.

Because of the lack of a control group and of reliable information about the incidence of urticaria or other allergic phenomena unrelated to penicillin in the recruit population, it was not possible to determine exactly the incidence of penicillin reactions. Most patients with allergic manifestations, particularly if mild, were afforded outpatient care and the diagnosis not recorded. Accordingly, arrangements were made to have all patients suspected of having penicillin or other allergic reactions brought to the laboratory regardless of whether or not admission was contemplated.

In order to obtain an estimate, all cases of urticaria or other hypersensitivity reactions which were referred to the laboratory or hospitalized during the courses of prophylaxis and within three weeks afterward were regarded as penicillin reactions. The results therefore indicate the maximum number of patients which might have been regarded as penicillin reactions by admitting physicians although many were not so regarded as evidenced by other admission diagnoses.

During the first course of prophylaxis there were thirteen cases (0.6 per cent) among men from the second regiment who received 50,000 units of penicillin daily, and thirteen cases (0.7 per cent) from the fourth regiment that received 500,000 units daily. During the second course of prophylaxis there were two (0.1 per cent) from the first regiment, seven (0.3 per cent) from the second regiment, and eight (0.4 per cent) from the fourth regiment. Thus, oral penicillin prophylaxis was attended by a very small incidence of untoward effects, there being no other complaints referable to administration. There was no evidence that those men who took both courses had been sensitized to the second course by the first.

All of the patients regarded as having reactions had mild urticaria. Thirty of the forty-three had previously had penicillin without reaction. Four men had previous reactions and had not applied for an excuse. Eight of the thirty men with a previous history of taking penicillin had parenteral penicillin within one week of beginning prophylaxis.

A past history of hypersensitivity reaction to penicillin was obtained on all patients with respiratory infections. Prior to the first prophylaxis in the three regiments, fifty-nine (3.4 per cent) of 1,719 admissions had previously experienced reactions. About one-half of these had been experienced following treatment for a respiratory or other infection for which the man had been hospitalized since his entry into the Navy.

The incidence of a past history of penicillin reactions in patients admitted during the several courses of prophylaxis and subsequent to them varied between 1.8 and 3.5 per cent with no indication that men who received prophylaxis had suffered any unreported reactions.

Penicillin Resistance of Streptococci.—All strains of beta hemolytic streptococci isolated were tested for sensitivity to penicillin.[13] All strains of Group A streptococci were sensitive to 0.04 unit of penicillin G.

DISCUSSION

These investigations were undertaken with the expectation that both doses of penicillin would alter the incidence of streptococcal infections. The major objective was to learn whether prophylaxis with 250,000 units of oral penicillin twice daily for a two-week period would accord prolonged interruption of an epidemic. If so, then intermittent prophylaxis could probably be used to control epidemic streptococcal infections at many military installations.

The 50,000 unit daily dose was, in a sense, a control program. The possibility was considered that any agent capable of markedly diminishing the number of streptococcal infections for a period of two or more weeks might so upset the epidemiologic balance as to greatly delay the return of disease rates to their former level. In addition, it was desired to compare the effectiveness of small doses of penicillin with that of larger doses during the period of actual prophylaxis.

It was recognized that the populations used should be relatively well isolated from possible reintroduction of epidemic strains of streptococci from sources outside of the men who received prophylaxis. During the planning of the study it was thought that this might be possible. Cut-backs in supporting personnel, with consequent inability to supply replicate services to each regiment of recruits and the large number of men hospitalized from all populations because of influenza in January made this impractical. Consequently, no attempt was made to enforce isolation of one regiment from the other or to limit the normal extraregimental contacts.

As had been suspected from the results of studies made in small groups of recruits, the 50,000 unit daily dose proved to be about as effective during the period of its administration as had doses ranging from 125,000 to 2,000,000 units daily in the large-scale studies which have been previously reported.[9, 10, 12] When compared to the abrupt cessation of streptococcal infections which occurred with the dose of 250,000 units twice daily, however, the effect of the 50,000 unit dose was gradual and incomplete. No explanation can be offered for the apparent greater effectiveness during administration of the 250,000 unit twice daily dose than was 1,000,000 units of oral penicillin twice daily as reported by Wannamaker and associates,[10] unless it reflects differences in the success of some of the recipients in escaping taking the tablets without detection. The diarrhea which followed the very large dosage in that study may have also played a role. During the present study it was known that many men missed one or more doses with some missing several in sequence. These misses were usually unavoidable and no effort was made to enforce the taking of every dose on such men. No man was detected attempting to avoid prophylaxis deliberately except those excused because of previous penicillin reactions.

By the end of the second week of prophylaxis with the 50,000 unit dose, streptococcal infection admission rates uniformly were one per 1,000 per week regardless of the preprophylaxis rate. When the dose was continued for an additional week, the rate remained at this low level. The probable mechanisms by which this small dose of penicillin achieves this degree of effectiveness have

not been fully defined. Previous studies have shown that it markedly reduces the numbers of men who acquire streptococci in their noses or throats during an epidemic. Men from the same companies who shared all activities with these men and who were not given prophylaxis continued to acquire the strains of streptococci which were causing the epidemic.[12] When given to an entire population already experiencing an epidemic, as in the present study, there is also the probable factor of reduced dispersion of streptococci by "dangerous carriers."[16, 17]

The rapid return of admission rates for streptococcal infections toward previous high levels after discontinuance of the 50,000 unit dose indicates that prophylaxis with doses of this order will probably have to be continuous to be very successful. The short-lived effect is believed to be a reflection of the failure of the 50,000 unit dose to alter markedly the number of carriers in the population. In Navy recruits the population turns over in approximately three months. Many of the graduates enter secondary training schools. Men from civilian life, who uncommonly harbor streptococci of the same types as are causing epidemics on the station, replace graduates in training. Continuation of prophylaxis for three months theoretically should achieve a clean recruit population if measures are taken to avoid their infection at the end of the program from other populations on the station. This would not apply to such secondary training schools as Navy service schools and hospital corps schools. Men arrive at these schools from three recruit centers, one on the east coast where the present studies were conducted, one in the Midwest, and one on the west coast. If streptococcal infections are occurring at any of the three recruit centers or at other activities from which these schools also draw students, infected men arrive and may start or continue an epidemic in the schools. An example occurred during the winter of 1952 to 1953 which is related to the results of the present study.

At the midwestern Naval Training Center all men arriving at the service schools between Dec. 5 and Dec. 18, 1952, were cultured. Ten per cent of ex-recruits arriving from the eastern center harbored Type 19 streptococci in their noses or throats. None of the men arriving from the other two centers harbored this type and few had positive cultures for any type. It was known that recruits at the midwestern center had few if any streptococcal infections prior to December of that year. On the other hand, cases of scarlet fever had occurred among service school students during November, and Type 19 streptococci were found to be prevalent in students seeking treatment for respiratory infections. Type 19 streptococci were found among recruits for the first time in December and became epidemic in the recruit population following the influenza epidemic in January, 1953. This apparent post-to-post transfer was in accord with observations made by others.[18, 19]

Administration of the 50,000 unit dose prophylactically to recruits at the eastern center would apparently not prevent such dissemination because of the slight effect on carriers of streptococci. On the other hand, a short course of the larger dose among service school students at the midwestern center would probably not have exhibited very lasting effects because of the continued introduction of men carrying virulent strains of streptococci in the nose or

throat. At this station continuous prophylaxis with the small dose after control of the epidemic with the larger dose might be necessary if it were desired to maintain a low incidence of streptococcal infections. For such reasons, a knowledge of the epidemiology of streptococcal infections in a population is a prerequisite to employment of prophylaxis and in selection of the prophylactic regimen to be used.

To evaluate fully the effects of the prophylaxis with the dose of 250,000 units of oral penicillin twice daily for fourteen days, a detailed analysis of the data was required. The effects can be summarized as follows:

1. If allowance is made for the latent period, admission of cases of rheumatic fever abruptly stopped.
2. Admission of patients with such clinical or laboratory evidences of streptococcal infection as scarlatinal rash, exudative tonsillitis or pharyngitis, dysphagia, cervical adenitis, leukocytosis, and/or positive throat cultures for Group A streptococci abruptly ceased or became uncommon.
3. The number of carriers, as measured by the percentage of men on duty with positive throat cultures for Group A streptococci, was markedly reduced.
4. Permanent alterations were produced in the prevalence of specific serologic types of streptococci among patients with streptococcal infections and in carriers.
5. Admission rates for such a patently nonbacterial infection as rubella were not influenced.

In view of the profound effects of this dose of penicillin on the incidence of streptococcal infections and on carriers of Group A streptococci, the recurrence of high admission rates and carrier rates within six weeks was quite disappointing. There are apparent reasons, however, for the short-lived effect. As previously mentioned, a Type 3 streptococcus, which was rarely encountered in recruits prior to prophylaxis, was responsible in large part for the epidemic which followed the first course of prophylaxis. Type 3 streptococci had been identified in other populations as early as late January. It was not found in 285 recruits newly arrived who were cultured in late February. Admissions with this type first occurred during the second week after prophylaxis and had been preceded by a few admissions caused by Type 19 streptococcus. At culture survey of about 25 per cent of the men in the regiment ten days after the end of prophylaxis all positive cultures were found within seven companies which were either present before prophylaxis or had entered training during it. Type 3 was found in more than one company. One man was identified as having entered a company while probably carrying this type following his discharge from the hospital. Twenty days after prophylaxis further spread had occurred of both Types 3 and 19 with a sprinkling of other types being found. The spread was in the older companies and additional men were found who had apparently entered some of these companies with positive cultures following discharge from the hospital or from other sources. Men from six new companies cultured at these surveys all had negative cultures although the companies contained men who had recently been hospitalized on the station.

These observations, although not extensive enough to be conclusive, strongly suggested that the postprophylaxis epidemic arose more from sources outside of the men who received prophylaxis than from the failure of the prophylaxis to eradicate carriers within the regiment. New recruits entering the regiment from civilian life could not be incriminated. One of the more probable outside sources appeared to be the transfer of men carrying streptococci into the companies following discharge from the hospital or from other regiments. In some companies nearly all the men cultured at surveys had been hospitalized one or more times and it was not uncommon to encounter men who had been on the station six or more months and who were still in the early weeks of training. Hospitalization was sufficiently prolonged in most instances to occasion the transfer of a man from his original company to a younger one. If therapy at the hospital failed to effect a bacteriologic cure in those men admitted because of streptococcal infection, or if the individual acquired additional strains prior to his discharge or in the reassignment unit en route to his new company, the man potentially was dangerous to his new company. The liberal use of dibenzylethylenediamine dipenicillin G plus the diminishing number of men returning from the hospital following initiation of the prophylaxis programs probably minimized this to some extent later in the spring. Another source was probably in the extracompany and extraregimental contacts of the individual men. As has also been previously pointed out, the fourth regiment shared the same mess hall with the second regiment and there were many other possible sources of individual contact between men of the fourth regiment and recruit or nonrecruit populations.

After reviewing the potential sources of reinfection it was more surprising that ten days after prophylaxis seventeen companies, and twenty days after, seven companies, still remained free than it was that a majority of the companies which had prophylaxis became reinfected. The term "reinfected" is believed to be proper since most of the companies were apparently free of streptococci ten days after prophylaxis and subsequently became involved. In some companies, however, the probability exists that prophylaxis failed to eradicate streptococci permanently from some carriers and these provided the focus from which the other men became reinfected. More than one type, however, was found in nearly all companies and pointed to more than one source of introduction.

In this connection it should be pointed out that it can hardly be expected that any regimen practical for use in prophylaxis will permanently eradicate streptococci from every carrier in the population. Some of the men on duty may have abnormalities of the paranasal sinuses or chronic middle ear infections. Eradication of the organism from such men is probably more difficult than from convalescent or chronic carriers who otherwise have no abnormalities of the respiratory passages.

Following the second course of prophylaxis, which was conducted more or less simultaneously in all three regiments, admission rates for streptococcal infections and carrier rates remained low. Presumably this was a seasonal

effect, although in the preceding year scarlet fever at this station reached its maximum incidence in May with a decline during June.[20] The declining population may have been a contributory factor. The lateness in the season of the prophylaxis, however, prohibits the drawing of any other conclusion from this second course than that both doses of penicillin again exhibited a marked effect on the incidence of streptococcal infections.

The results of these investigations cannot be compared directly to the results of other large-scale prophylaxis studies with oral penicillin.[9, 10] With one exception,[12] those studies were carried out on a controlled basis in which men receiving prophylaxis remained in intimate contact with untreated men. It was to be expected that a rapid return of streptococcal infections would occur once prophylaxis ceased. In the only other study where entire regiments were treated,[12] the majority of men received 125,000 units daily for a thirty-day period. Since this dosage was inadequate to eliminate the organism from a high percentage of carriers and did not wholly suppress infections, the return of epidemic rates was not surprising. As one-third of the population had been replaced during the prophylactic program, the increase in rates was delayed more than was observed as a result of the 50,000 unit dose used for only two or three weeks in the present study.

One of the more interesting and significant observations was the marked effectiveness of penicillin prophylaxis in reducing admissions for rheumatic fever. The results in the first and second regiment were as expected. In the fourth regiment, where no cases occurred following the first course of prophylaxis despite recurrence of epidemic streptococcal infections, the results were surprising. One possible explanation may be that of Rantz and his co-workers,[21] who were of the opinion that multiple streptococcal infections occurring in close sequence were important in the pathogenesis of rheumatic fever in military personnel. Reinstitution of prophylaxis after an interval of only six weeks may have prevented this from occurring to any great extent. The decline cannot be attributed to improved therapeutic regimens at the hospital since less than one-half of rheumatic fever patients were treated for the initiating streptococcal infection.

Wannamaker[10] has mentioned the desirability of intermittent prophylaxis over continuous prophylaxis. This is to be emphasized. Acceptance of prophylaxis by the populations in which studies have been carried out has been conditioned by interested observers, close supervision, and an effect apparent to the men themselves, since penicillin has been instituted when streptococcal infections posed a problem to men trying to finish their training. Continuous prophylaxis in a population such as recruits, however, involves administration of the agent to companies who enter training after the epidemic of streptococcal infections has subsided. Under these conditions men can see no advantage to be gained, and soon try to avoid taking the tablets as a nuisance or because they are ineffective in preventing the nonbacterial respiratory infections which are prevalent in new recruits. Some even blame such infections on the tablets. Moreover, personnel administering the program soon lose interest after the

admission rates fall. Under these conditions, oral prophylaxis is wasteful and expensive. It is such spotty administration that will promote the development of penicillin-resistant bacteria in the population and under which a penicillin-resistant streptococcus is most likely to emerge, if there is such a strain. Strains of resistant Group A streptococci were not found during the present study. Parenterally administered depot penicillin providing prolonged blood levels may prove to be more practical than oral penicillin for continuing prophylaxis.[23]

Hypersensitivity reactions and other untoward effects of prophylaxis occurred in such a small percentage of the men given oral penicillin that the problem was negligible in view of the benefits derived in the control of streptococcal infections and rheumatic fever.

The cost of prophylaxis was neglible in comparison with the cost of medical care necessary for patients with streptococcal infections during periods when it was not given. At the time the study was made, the cost of 500,000 units daily for two weeks was approximately seventy cents per man. The prevention of one case of rheumatic fever with its prolonged hospitalization and potential disability benefits saved more than enough money to have provided the penicillin for complete suppression of streptococcal infections in a population this size for an entire winter. The results suggest, however, that it is not necessary to provide complete and continuous suppression of streptococcal infections to prevent rheumatic fever and that intermittent prophylaxis, if administered to all involved populations, is a practical method of control. A paucity of admissions from a population cannot be taken to mean that it is not involved.[13]

The remarkable effectiveness of the oral dose of 250,000 units twice a day suggests that it may prove highly useful as a tool for estimating to what extent streptococcal infections are etiological in mixed epidemics of streptococcal and other infections. If there is not a marked change in the disease picture within forty-eight hours after institution of this dosage, then a majority of infections are probably of nonstreptococcal etiology.

SUMMARY AND CONCLUSIONS

Entire regiments of Navy recruits experiencing epidemic streptococcal infections and other respiratory diseases were given oral penicillin prophylaxis. One regiment, numbering about 2,000 men, received 250,000 units of penicillin twice daily for two weeks. This dose abruptly terminated admissions for streptococcal infections, markedly reduced the percentage of men in active training who harbored Group A streptococci in the throat, and caused a persisting alteration in the prevalence of specific serologic types of streptococci. Epidemic admission rates recurred gradually over a six-week period but there was evidence that the recurrence was due to reinfection of the regiment from other sources on the base more than from failure of prophylaxis to reduce carrier rates or through entry of new recruits from civilian life. When epidemic rates recurred, the same dose was given for a period of three weeks with essentially similar results.

Concurrently, a second regiment of the same size was given prophylaxis with a single 50,000 unit penicillin tablet daily for the same periods of time. This dose markedly reduced admission rates for patients with streptococcal infections but had comparatively little effect on carriers. Recurrence of epidemic rates occurred almost immediately on termination of prophylaxis. A third regiment was also given the 50,000 unit daily dose during the second course of prophylaxis in the other two regiments. Admission rates for streptococcal infections had continued high throughout the period preceding prophylaxis and the results were essentially similar to those obtained in the other regiment given this dose. It seemed obvious that this dose was too small for initial control of an epidemic but that it would prove adequate in preventing the initial outbreak or recurrence of an epidemic if given continuously.

Both doses of penicillin had a marked effect on admission of patients with rheumatic fever from the regiments. If allowance is made for the latent period, the first course of 250,000 units twice daily terminated admissions for this sequel of streptococcal infection. No further cases occurred in eighteen weeks of observation despite the brief recurrence of epidemic streptococcal infections in the men. It appears possible that intermitent prophylaxis with this dose of penicillin may completely control epidemic rheumatic fever without completely controlling streptococcal infections if it is given to all involved populations. The 50,000 unit daily dose reduced but did not terminate admissions for rheumatic fever. Neither dose had any effect on the admission of patients with nonbacterial infections.

Hypersensitivity reactions to oral penicillin occurred in less than 1 per cent of the populations receiving prophylaxis and there was no evidence that prophylaxis sensitized men to later penicillin. The risk of a reaction was sufficiently low to render it negligible in comparison to the risk of men having serious complications and sequelae of streptococcal infection during an epidemic. There was no evidence of increased resistance to penicillin of the streptococci isolated during these studies.

Our most sincere appreciation is due Captain Clifford A. Fines, United States Navy, Commander of the Naval Training Center, Bainbridge, Maryland, and to the officers and men of the Naval Training Center, Recruit Training Command, and the Medical Department, for their cooperation and assistance in making this investigation possible. In particular we would like to express our appreciation to Captain Frederick Wolsieffer, United States Navy, Commanding Officer; Commander George Sotos, Executive Officer; and Lieutenant Commander J. C. Gilmore, Training Officer, of the Recruit Training Command. Our especial thanks are also due Captain Tilden I. Moe, MC, United States Navy, Senior Medical Officer of the Naval Training Center and to the Commanding Officer of the United States Naval Hospital and his staff for their many contributions.

Our greatest gratitude is due the following personnel of the Hospital Corps of the United States Navy: CWO Clifford D. Shick, HC, United States Navy, HMC M. J. Ferenchek, HM1 P. M. Polaski, HM2 J. M. Mosley, HM2 H. M. Cohen, HM3, J. K. Locke, HM3 T. C. Black, HM3 J. J. Forsythe, HM3 V. B. Sonsini, HM3 W. A. Sickel, HM3 J. R. Seitchek, HM3 R. H. Klemm, HM3 C. A. Ryberg, HN R. E. Whispell, HN A. T. Major, HN A. J. Tobin, HN H. Howell, HN D. Sherman, and PN H. Huff. Without their loyalty and willingness to work long and tedious hours the study would have not been possible, and our thanks are small return for their valuable services.

We would also like to acknowledge the assistance of the many members of the several departments of the Naval Medical Research Unit No. 4 for their assistance during the study and in preparation of the manuscripts.

These studies have been coordinated with those made in other branches of the armed forces through personal communications with Dr. Charles H. Rammelkamp, Jr., and other members of the Commissions on Acute Respiratory Diseases and Streptococcal Infections of the Armed Forces Epidemiological Board. The results were reported at a meeting of the ad hoc Committee on Prophylaxis of Streptococcal Infections, Commission on Streptococcal Infections, Washington, D. C., in October, 1953. Many of the ideas were a result of these consultations over a period of several years and are gratefully acknowledged. Clayton G. Loosli, M.D., Department of Preventive Medicine, University of Chicago, has also contributed ideas and advice in his role of consultant to the Naval Medical Research Unit No. 4.

ADDENDUM

Since submission of these papers, studies made at Sampson Air Force Base on prophylaxis of streptococcal infections with oral penicillin have been published (Bernstein, S. H., et al., J. LAB. & CLIN. MED. 44: 1-13, 1954). Results with a dosage of 250,000 units of oral penicillin twice daily for ten days were remarkably similar to those of the present study with the same dose. Results with this dose for a period of five days were scarcely better than obtained with a dose of 50,000 units in the present study. The results of the present study make it doubtful that treatment of new additions to a recruit population will delay the recurrence of the epidemic beyond what might be expected from continued prophylaxis in any considerable segment of the population. The over-all incidence of hypersensitivity reactions to penicillin in the two studies involving more than 40,000 men were also similar. Serum sickness type or severe reactions, however, were not encountered among the Navy recruits on either of the two doses of penicillin used.

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THE PATHOGENESIS OF THE ANEMIA OF CHRONIC LEUKEMIA: MEASUREMENT OF THE LIFE SPAN OF THE RED BLOOD CELL WITH GLYCINE-2-C^{14}

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ANEMIA not resulting from extravascular blood loss can arise either from a decrease in the rate of production of red blood cells, a decrease in the life span of the red blood cells, or a combination of these two factors. For many years it has been held that the anemia of leukemia results from a crowding out of the erythropoietic fraction of the bone marrow by leukemic tissue, resulting in a decreased rate of formation of red cells. Often the histologic appearance of the bone marrow would tend to confirm this opinion. However, in 1949, investigations in this laboratory showed surprisingly, but conclusively, that in most cases of chronic leukemia, the rate of production of red blood cells was normal or even greater than normal.[1, 2] Since these patients are in many instances anemic by blood volume standards, it was postulated that the red cell life span was decreased in this disease. Direct measurement of the life span of the red cell in several patients with chronic leukemia[3, 4] indeed did show a shortened life span. Similar findings based on the Ashby differential agglutination technique were reported from several other laboratories.[5-7] However, the Ashby method measures only the survival of normal donor cells in the patient.

With the demonstration that glycine is a specific precursor for the 4 nitrogen atoms and 8 of the 34 carbon atoms of the porphyrin moiety of hemoglobin, it became possible to measure the life span of the red blood cell in its natural environment.[8] The present paper is a more detailed and extended report on the life span of the red blood cell in eight patients with chronic leukemia as measured by the carbon-14 labeled glycine method. Three of these patients have been briefly reported in our original paper.[4]

METHODS

Each patient was given approximately 100 μc of carbon-14 as glycine-2-C^{14} (8.2 mg. of glycine) intravenously. Blood samples were obtained at frequent intervals. The blood was centrifuged, and the plasma removed. The hemoglobin was extracted by a modification of the method of Drabkin,[9] in which the cells were washed three times with physiologic saline. When a definite layer of white blood cells was present, it too was removed. After the third washing, the cells were laked in distilled water. Toluene was added and the mixture was shaken, and then allowed to stand overnight. The stroma and the lipid-containing toluene layers were separated by centrifugation. From the intervening clear layer, an aliquot of the hemoglobin solution was aspirated. This was then dried in vacuo and combusted to carbon dioxide with a chromic-sulfuric acid mixture.[10] The specific activity of the carbon

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These studies were supported by U. S. Public Health Service Grant C-1440 (C2), and the labeled glycine and the equipment used were provided by the Atomic Energy Commission.
Received for publication March 1, 1954.

dioxide was measured in a 100 ml. ionization chamber, at atmospheric pressure, using a vibrating reed electrometer and a recording potentiometer.[11] Analysis of the dry hemoglobin powder for iron content showed that this material was approximately 95 per cent hemoglobin.

RESULTS

Eight patients, three with chronic lymphatic leukemia and five with chronic myelogenous leukemia, were studied. The hematological course and therapy, together with graphs for the specific activity of the hemoglobin, are presented in Figs. 1-16. The red cell life span was determined from the graph of the specific activity of the hemoglobin by the method of Shemin and Rittenberg.[8] The graphs for the red cell life spans are similar to those which have been observed in normal and in pathologic states in man and animals.

Chronic Lymphatic Leukemia.—

Patient 1: F. E., a 40-year-old white man (Fig. 1). This patient was studied during a period when the leukemic process was relatively well stabilized, testosterone being the only therapy given. A normal red cell life span of 113 days was found (Fig. 2). Throughout the course of the study the spleen was palpable at the costal margin, but the patient was not anemic.

Patient 2: E. H., a 63-year-old white man (Fig. 3), was studied when the white blood cell count was showing a slow, progressive rise, while at the same time the red cell count and hemoglobin showed a progressive decline and the frequency of radioactive phosphorus therapy was increasing. The red cell life span was 102 days, only slightly, if at all, shorter than normal (Fig. 4). Throughout the period of the study the spleen was palpable two to three fingerbreadths below the costal margin.

Patient 3: J. R., a 68-year-old white man (Fig. 5), was studied during the terminal phase of his disease. Under radioactive phosphorus therapy, the white cell count declined, but the red cell count also continued to decrease. The spleen was very large, extending to the right lower quadrant. The red cell life span does not fit the pattern observed in the first two patients or that observed in normals with nitrogen-15, but if the logarithm of the specific activity of the hemoglobin after nine days is plotted as a function of time, a straight line is obtained (Fig. 6). This can be interpreted as indicating random destruction of labeled red cells.

The finding that the decreasing portion of the curve of the specific activity of the hemoglobin can be fitted to a straight line when plotted on semilogarithmic paper indicates that a fixed fraction of the circulating labeled cells are destroyed per day irrespective of the length of time that the cell has already lived. This interpretation would indicate that this patient's cells have a mean life span of eighteen days and indicates a hemolytic process. (The mean life span under these conditions is 1.44 times the half-life). This is only approximate, since four transfusions were given during the period of study. It should also be noted that this patient achieved a peak hemoglobin specific activity approximately three times as high as any other individual, indicating a greater utilization of the radioactive glycine for hemoglobin formation than in the other patients.

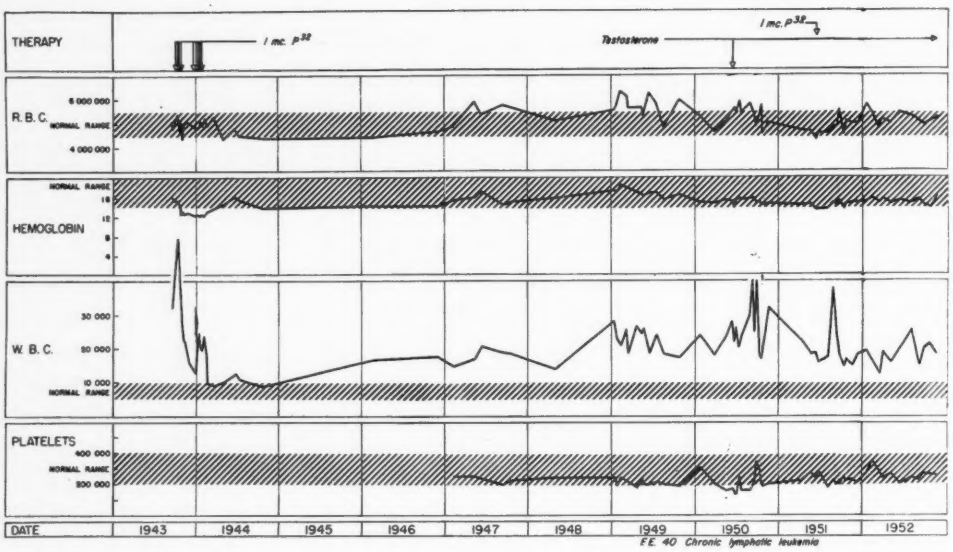

Fig. 1.—Hematological course of a 40-year-old white man (F. E.) with chronic lymphatic leukemia.

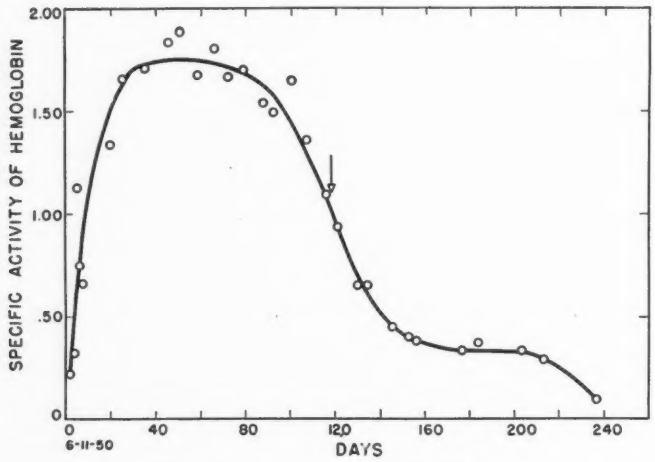

Fig. 2.—Red cell life span in F. E.

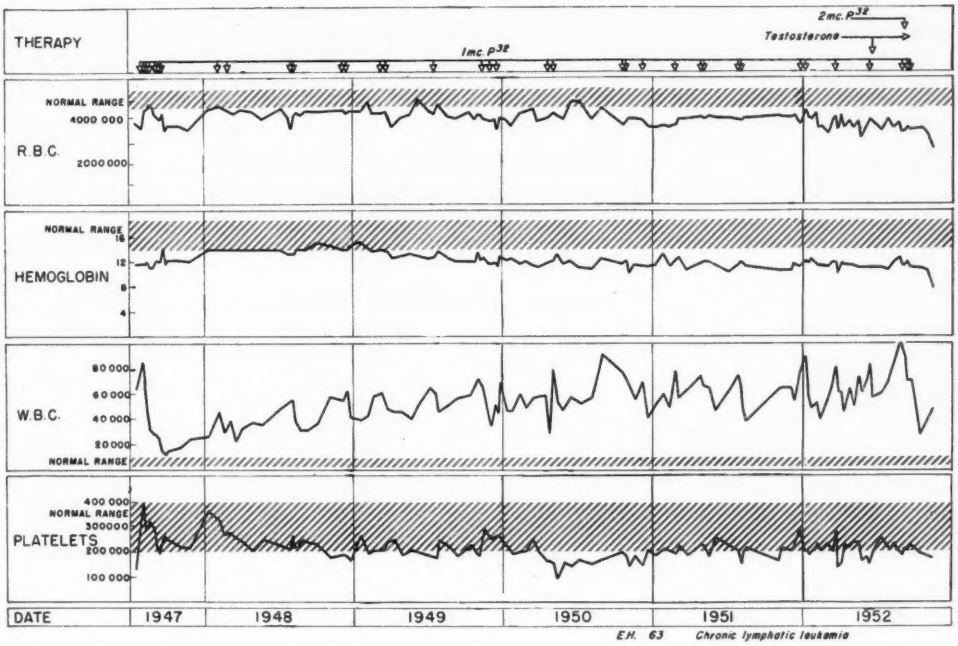

Fig. 3.—Hematological course of a 63-year-old white man (E. H.) with chronic lymphatic leukemia.

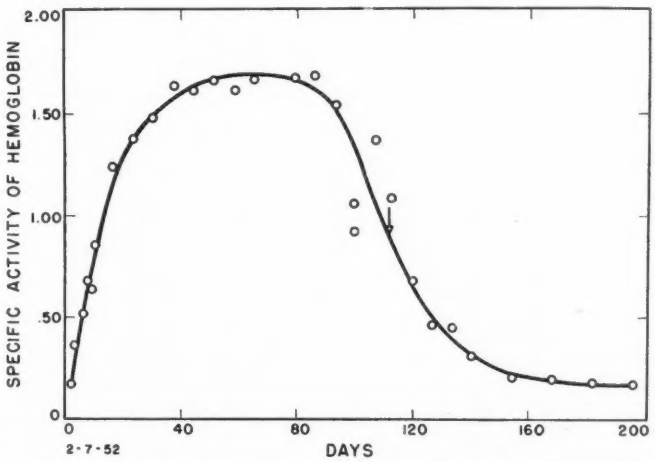

Fig. 4.—Red cell life span in E. H.

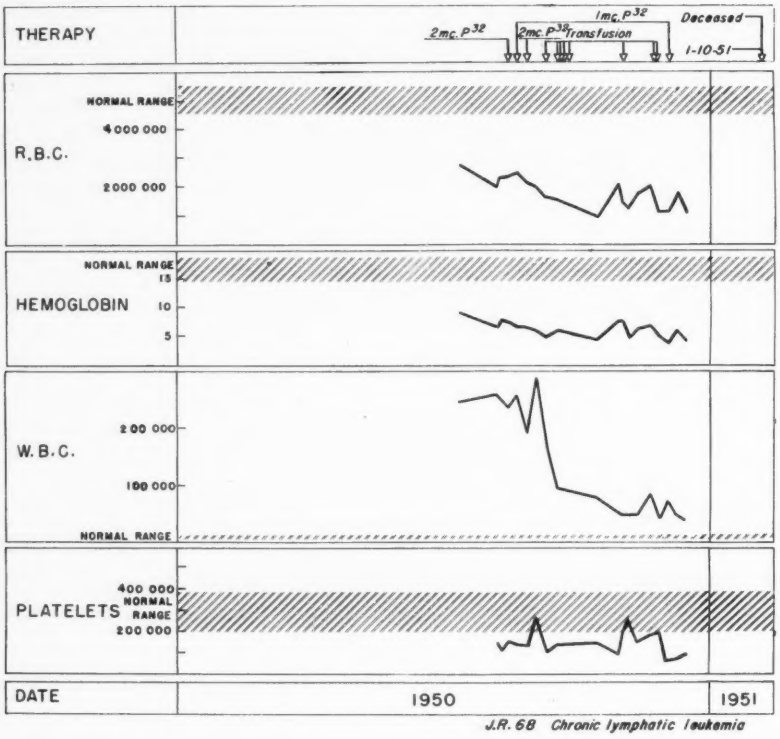

Fig. 5.—Hematological course of a 68-year-old white man (J. R.) with chronic lymphatic leukemia.

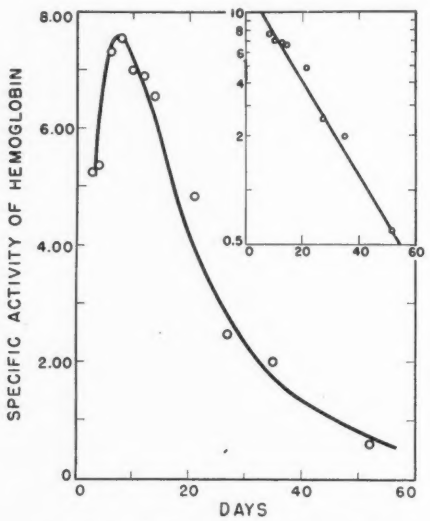

Fig. 6.—Red cell life span in J. R.

Chronic Myelogenous Leukemia.—

Patient 4: P. N. (Fig. 7) was a 56-year-old white man in the chronic myeloid leukemic phase of polycythemia vera.

Patient 5: W. F. (Fig. 9) was a 24-year-old white man with chronic myelogenous leukemia of two years' duration at the time of this study.

Patient 6: E. P. (Fig. 11) was a 32-year-old Negro with chronic myelogenous leukemia of three months' duration at the time of this study.

Patient 7: G. J. (Fig. 13) was a 52-year-old white woman with chronic myelogenous leukemia of six months' duration at the time of this study.

Patient 8: G. W. B. (Fig. 15) was a 64-year-old white man with chronic myelogenous leukemia of two years' duration at the time of this study.

Patients 4, 5, 6, and 7 had finite red cell life spans of 70 to 83 days (Figs. 8, 10, 12, and 14, respectively). Patient 8 had a finite red cell life span of 100 days (Fig. 16). Although this is not as short as the others, it is probably slightly shorter than normal.

With the exception of Patient 8, these individuals with myelogenous leukemia had large, palpable spleens generally extending below the umbilicus at the time of these studies.

In Patient 2 with chronic lymphatic leukemia and Patient 5 with chronic myelogenous leukemia, there is noted a slow continual rise in the specific activity of the hemoglobin during the period of the twentieth to sixtieth day after administration of the labeled glycine. This probably results from some depression of the rate of red cell production due to the P-32 administered just before these studies were begun. As a result of this P-32 therapy, there is a transient decrease in the rate of production of red cells. However, the red cells already formed will continue to be destroyed as they reach the end of their life span—thus more red cells will be removed from the circulation per day than are produced per day. The net effect is an increase in the relative concentration of red cells containing carbon-14.

In an attempt to correlate the red cell life span with other clinical data, Table I shows the reticulocyte count, serum bilirubin, fecal urobilinogen, and rates of red cell production as measured with Fe^{59}.[2] This table shows that

TABLE I

	RETICULOCYTES (PER CENT)	FE TURNOVER* (MG./KG. OF BODY WT./DAY)	FECAL UROBILINOGEN (MG./DAY)	SERUM BILIRUBIN (MG. PER CENT)
Lymphatic				
1. F. E.	0.2-1.2	0.33	110	0.083
2. E. H.	0.1-1.2	0.26	182	0.31
3. J. R.	19-26	- -	252	0.2
Myelogenous				
4. P. N.	0.2-18.5	0.55	487	
5. W. F.	1.3-8.5	1.32	203 (2/21/51)	
			47 (3/26/51)	
6. E. P.	0.6-10.0	- -	- -	
7. G. J.	1.6-5.2	- -	- -	
8. G. B.	1.6-3.2	0.26	24	

*Fe turnover = The iron turnover as measured with Fe^{59} expressed as milligrams per kilogram of body weight per day for hemoglobin formation (normal, 0.26 milligrams per kilogram per day).

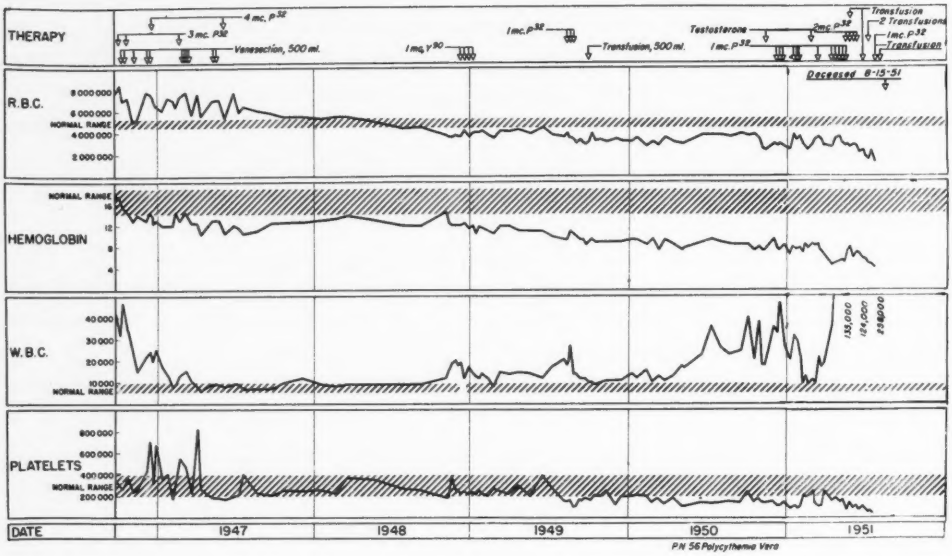

Fig. 7.—Hematological course of a 56-year-old white man (P. N.) in the chronic myeloid leukemic phase of polycythemia vera.

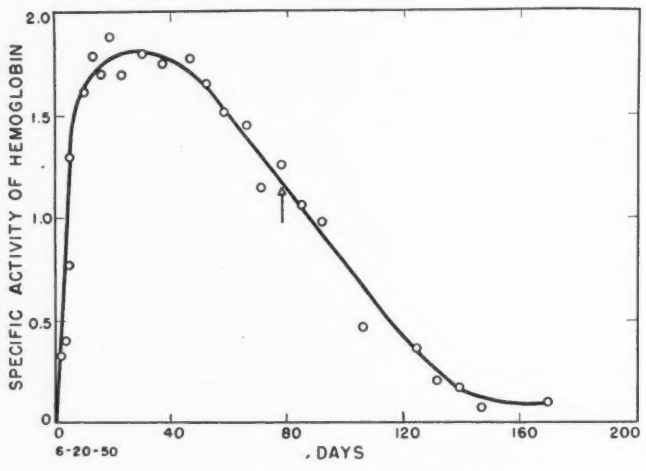

Fig. 8.—Red cell life span in P. N.

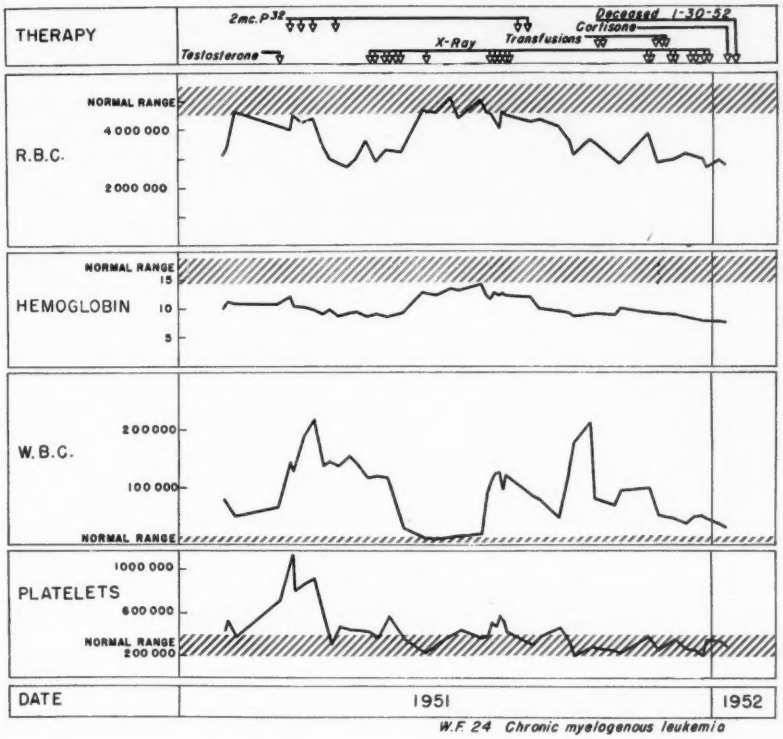

Fig. 9.—Hematological course in a 24-year-old white man (W. F.) with chronic myelogenous leukemia.

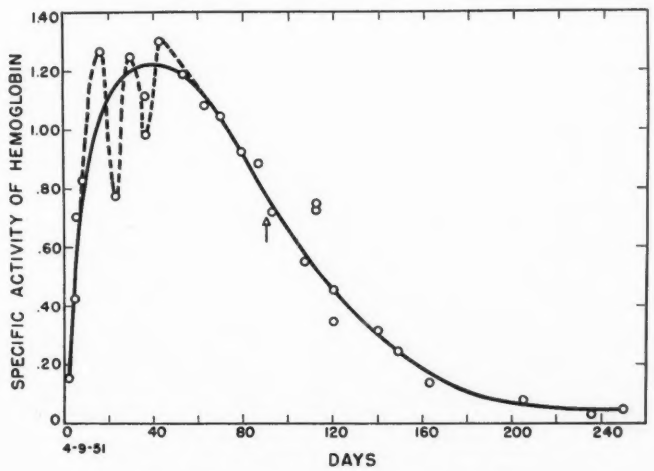

Fig. 10.—Red cell life span in W. F.

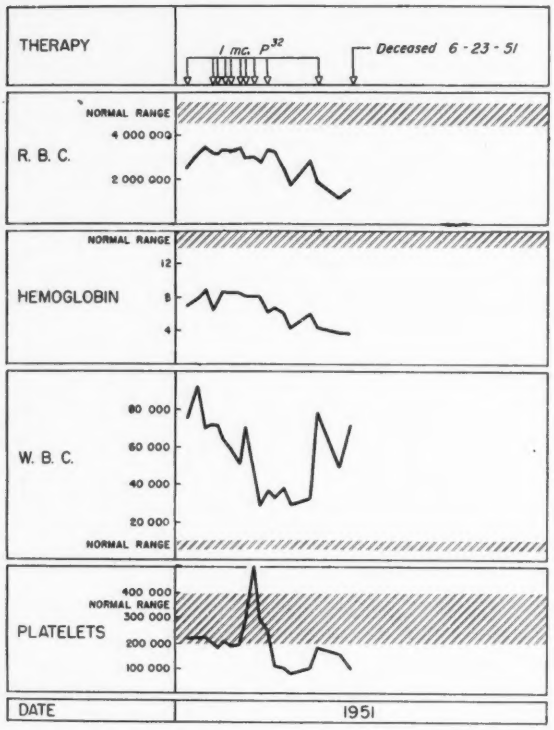

Fig. 11.—Hematological course in a 32-year-old Negro woman (E. P.) with chronic myelogenous leukemia.

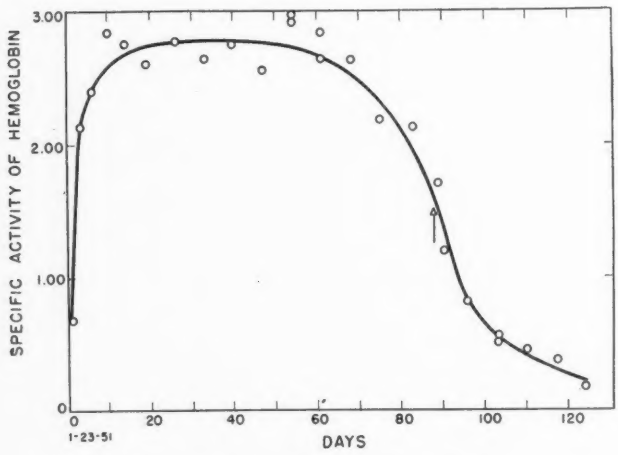

Fig. 12.—Red cell life span in E. P.

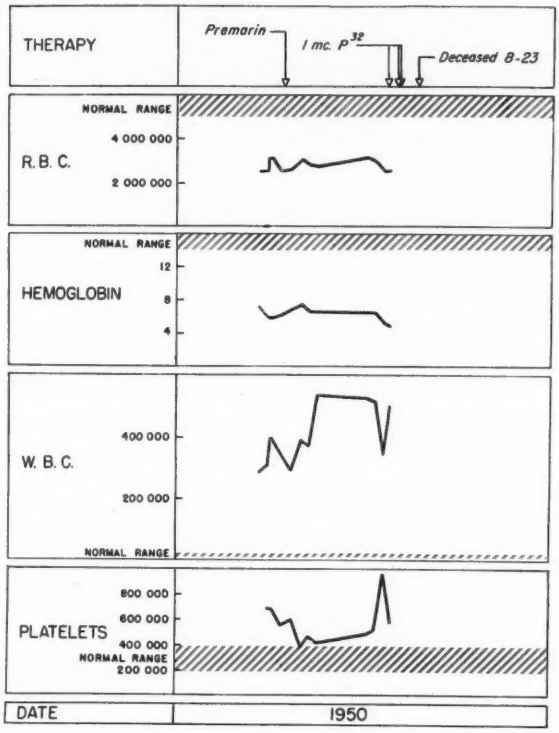

Fig. 13.—Hematological course in a 52-year-old white woman (G. J.) with chronic myelogenous leukemia.

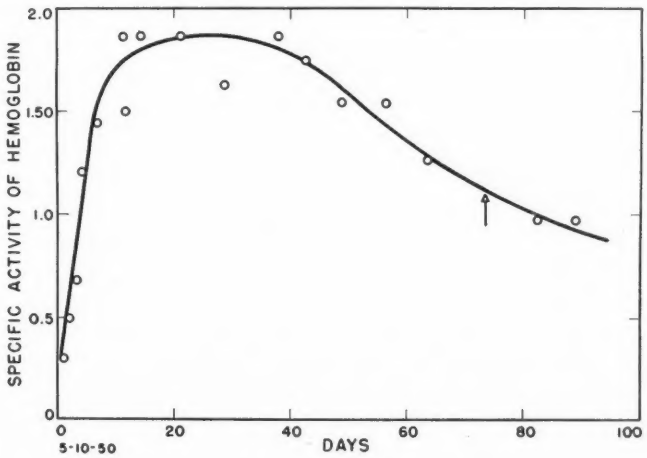

Fig. 14.—Red cell life span in G. J.

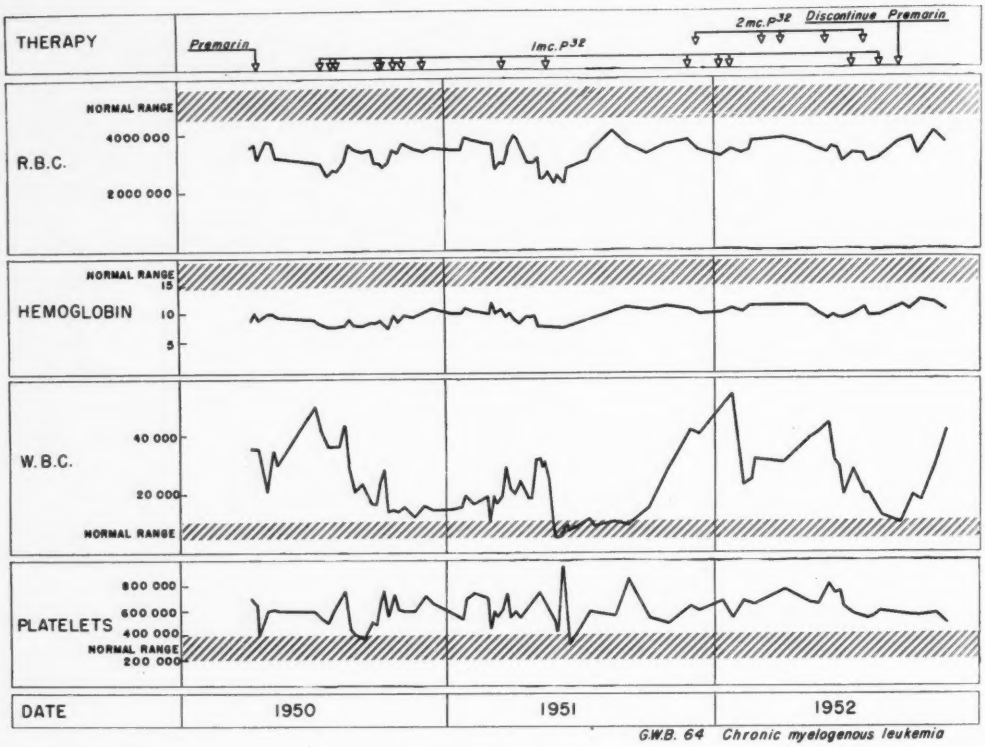

Fig. 15.—Hematological course in a 64-year-old white man (G. W. B.) with chronic myelogenous leukemia.

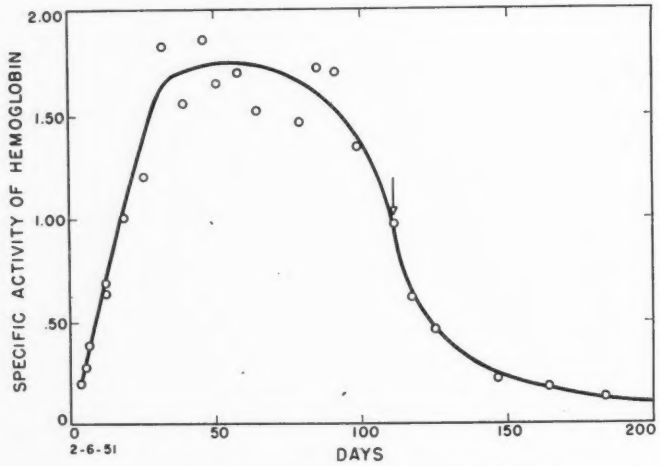

Fig. 16.—Red cell life span in G. W. B.

of the three patients with lymphatic leukemia only one had a reticulocytosis and this was the only patient with chronic lymphatic leukemia who had a short red cell life span with a pattern corresponding to random destruction of red cells. All five patients with chronic myelogenous leukemia had a reticulocytosis and all had a decrease in the life span of the red blood cell. In the three patients with chronic lymphatic leukemia the serum bilirubin was normal; it was not measured in the group with myeloid leukemia. The fecal urobilinogen excretion was measured in three of the five patients with chronic myelogenous leukemia. In one, it appears to be elevated, and in two, it is low. In two patients (Cases I and 2) with chronic lymphatic leukemia the fecal urobilinogen excretion appears to be within normal limits, while in Patient 3 there is some increase, although it is not marked. Patient 3 with chronic lymphatic leukemia, who had a very short red cell life span, also had the highest urobilinogen excretion in this group of patients. Therefore, of these laboratory determinations, the one that correlated best qualitatively with the measurement of red cell life span was reticulocyte count, but this alone could not be used to predict the red cell life span.

It has been previously reported from this laboratory that in patients with chronic leukemia the red cell production as measured with Fe^{59} is usually normal or greater than normal. In the present studies, red cell production has been measured in two cases of chronic lymphatic leukemia (Cases 1 and 2), and in three patients with myelogenous leukemia (Cases 4, 5, and 8). In both the cases with lymphatic leukemia, the red cell life span was normal and the red cell production rate was normal. In one of the cases of myelogenous (Case 8), the red cell production was normal; and in two (Cases 4 and 5), greater than normal. It would be anticipated that in the case of three patients with myelogenous leukemia and the case with lymphatic leukemia, who had a very short red cell life span and who died before this determination could be done, red cell production rates would also have been normal or greater than normal.

Using multiple scintillation counters placed over spleen, liver, and bone marrow, Case 1, with chronic lymphatic leukemia who was not anemic was studied with Fe^{59}, and showed a pattern of iron turnover in these organs that was normal. In six other cases not reported in the present study, it was found that there was a definite secondary rise in the counting rate over the spleen in three patients; in two the rise was slight, and in one there was no such rise. This secondary rise has been interpreted to indicate premature destruction of erythrocytes in the spleen.[12, 13] Of these seven patients with in vivo radioiron studies, five had a normal total red cell volume. In three of these five patients with normal total red cell volume, there was evidence for splenic erythroclasis. The two definitely anemic patients both showed a secondary spleen rise indicating splenic erythroclasis.

In Patient 5, with chronic myelogenous leukemia, and a shortened but finite red cell life span, the in vivo radioiron study showed a rapid early rise in the counting rate over the spleen site; this rate was maintained at a high level during the course of observation. This early rise in counting rate was

interpreted as indicating splenic production of red cells, usually present in chronic myeloid leukemia, while the failure of the counting rate to fall may be interpreted as being due to splenic erythroclasis. This case is characteristic of all four other cases of myelogenous leukemia, not the subject of the special study reported in this paper, studied with radioiron.[12, 13]

DISCUSSION

In the first two cases of lymphatic leukemia, who were not anemic, the red cell life was normal; but in the third patient, who was in the anemic, terminal phase, the red cell life span was considerably shortened and had a pattern of random destruction. The anemia, then, in Patient 3 was probably due in large part to this hemolysis, and is similar to that which has been observed in sickle cell anemia[14] and in experimental animals after hemorrhage.[15, 16] In this case, there was classical evidence of a hemolytic process as demonstrated by marked reticulocytosis and increased fecal urobilinogen excretion, although the increase in urobilinogen excretion was smaller than would be expected as calculated from the life span of the red cell and the total red cell volume. In the five patients with chronic myelogenous leukemia, the red cell life span was shortened, but finite.

In the absence of bleeding, anemia may be due either to a decreased production of red cells or a decreased life span, or a combination of these two factors. With the demonstration by radioiron that the bone marrow is producing a normal or greater than normal number of red blood cells and that in one of three cases of chronic lymphatic leukemia and in the five cases of chronic myelogenous leukemia, the red cell life span is shortened, the pathogenesis of the anemia in the absence of hemorrhage is clearly, then, a result of the shortening of the life span of the red blood cell. However, the bone marrow in these individuals, although producing a normal or greater than normal amount of red cells per day as measured with radioiron, is still not producing a sufficient number to compensate for the decreased red cell life span, and furthermore, is not responding to this stimulus with an increase in production of the magnitude to which the bone marrow appears to be capable. Patients with polycythemia vera[2] and hereditary hemolytic anemia[18] appear to be able to increase the rate of production of red cells about six to nine times, while in leukemia, the maximum observed increase is approximately fourfold. In this sense, in leukemia the bone marrow is abnormal with respect to erythropoiesis. This does not, however, negate the conclusion that the fundamental defect leading to anemia is a decrease in the life span of the red blood cell.

Utilizing the Ashby differential agglutination technique in three cases of chronic lymphatic leukemia, Brown and co-workers[6] found two with finite, normal red cell life spans and one with shortened random red cell destruction; Sheets and co-workers[17] studied one case and found random red cell destruction with a shortened red cell life span; while in nine cases studied by R. Berlin,[5] five may be interpreted as indicating a finite, almost normal red cell life span, two cases showed random destruction with markedly shortened red cell life span, and in two cases the follow-up was incomplete. In agreement

with Brown, Elliott, and Young,[6] and in partial agreement with R. Berlin,[5] we have found that the red cell life span may be either normal or very short in chronic lymphatic leukemia, and the destruction process may be random, suggesting splenic destruction, as in Patient 3, or, as in Patients 1 and 2, the cells may have a normal life span or a slightly shortened but finite life span (Patient 2), suggesting in this instance defective cells. In Patient 3, with a large spleen, the destruction of red cells was random, but in Patients 1 and 2, although the spleen was enlarged, the red cell life span was finite. Presumably, Patient 3 presents the type of situation in which splenectomy might temporarily benefit the patient.

R. Berlin,[5] using the Ashby differential agglutination technique in fifteen cases of chronic myelogenous leukemia, found red cell 50 per cent survival times varying from 5 to 63 days, or red cell life spans of 10 to 126 days; however, in only two cases was the 50 per cent survival greater than 44 days, and in the majority of cases it was less than 30 days. In these fifteen cases the graphs indicate a finite and shortened red cell life in nine cases, finite and normal in two, random destruction with shortened red cell life in three, and in one case it is difficult to decide. Thus, in general, his reported survival times are shorter than would be predicted from the present study. This apparent difference may either reflect different types of patients or be the result of different methods. This suggests that the Ashby technique may not be entirely satisfactory, since it determines the survival of normal cells in an abnormal environment, while the labeled glycine method determines the life span of the red cell in its natural environment.

The red cell life span may be shortened in two ways, the first due to some intrinsic defect in the red cell, the second to some extracorpuscular factor producing hemolysis. The defect in the first instance may result in a red cell life span that is finite and shortened or to a random type of destruction, while in the second case the destruction will be random. An example of the first type of defect is seen in the shortening of the red cell life span after acute hemorrhage[13, 14] and in the random destruction of red cells in sickle cell disease, while that of extracorpuscular origin is seen in the hemolysis resulting from mismatched transfusions.

The finding in all five cases of chronic myelogenous leukemia that the red cell life span was finite and shortened leads to speculation that the basic defect is inherent in the red cell and not due to some extracorpuscular factor, such as hypersplenism, which should produce a random type of destruction.

The Ashby differential agglutination studies of R. Berlin[5] would indicate that the defect in chronic myelogenous leukemia is extrinsic, since normal red cells have a shortened life span, while the present studies would indicate that the defect is intrinsic since the red cells have a finite but shortened life span. In one of the three cases of lymphatic leukemia, such a random type of destruction was seen, suggesting that in that instance hypersplenism or some other extracorpuscular factor was producing the anemia, although even in this instance it is possible for the defect to be within the red cell.

CONCLUSION

1. The red cell life span was approximately 18 days in one case of lymphatic leukemia and 102 and 113 days, respectively, in two additional cases. In the former there was random destruction, while in the latter two, the life span was finite and normal, or slightly shorter than normal.

2. The red cells had finite life spans of 70 to 100 days in five cases of myelogenous leukemia, suggesting an intrinsic defect in the red cell in chronic myelogenous leukemia.

3. The anemia of leukemia, when not due to hemorrhage, is the result of shortening of the life span of the red cell and not due to crowding out of the red cell precursors in the bone marrow.

4. Reticulocyte counts correlated negatively better with red cell life span than did serum bilirubin or fecal urobilinogen, but none of these tests can predict with any accuracy the degree of shortening of the life span of the red cell.

ADDENDUM

Since the preparation of this manuscript, Weinstein and LeRoy (J. LAB. & CLIN. MED. 42: 368, 1953) reported that, as measured with Cr^{51}; the life span of the red cell was normal in one case, approximately two-thirds of normal in another case and approximately one-fourth of normal in a third case of chronic lymphatic leukemia.

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THE CAUSE OF RISE IN SEDIMENTATION RATE AT LOW LABORATORY TEMPERATURE

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MOST authors believe that a high temperature increases and a low one decreases the red cell sedimentation rate (S.R.). Temperature, however, has been shown to exert an inconstant effect on S.R., so that in many cases a higher S.R. is recorded at the lower temperature when the test is performed simultaneously in high and low temperatures.[1] Weingarten[1] also observed that a higher S.R. in the colder temperature appears to be associated with damage to the parenchyma of the liver.

Little study has been made of the accelerating action of low temperatures on S.R. Buttaro and Tosti[2] believe that it is the result of auto-agglutination in the cold due to instability of the isoelectric point in persons whose humoral equilibrium has been disturbed. According to these authors the accelerating factor would be most prevalent at low temperatures, while cold agglutination is extremely rare. This paper presents the results of an investigation of the cause of the paradoxical behavior of S.R.

MATERIAL AND METHOD

For this investigation cases showing a higher S.R. in the colder temperature were selected. Sedimentation rate was determined by Westergren's technique at temperatures of 99° and 44° F., in an incubator and a refrigerator, respectively. Readings were taken every ten minutes for a period of one hour. Cases having a higher S.R. at 44° F. (more than 10 per cent) have been regarded as showing a higher S.R. in the colder atmosphere and vice versa.

Fractionation of plasma proteins was carried out and it was possible to isolate the fraction responsible for the accelerated S.R. in a cold temperature. This fraction, when added to blood exhibiting a higher S.R. in the warmer chamber, changed the blood so that it showed a higher fall in the colder chamber.

RESULTS

Fractionation of plasma proteins was done by the salting-out technique. The influence of an individual protein fraction was determined either by excluding it from the experiment, thus noting the effect of its absence, or by adding it and seeing the effects of its presence.

To find out the influence of fibrinogen, sedimentation was effected in the serum and the plasma of the same individual. Five milliliters of blood was collected as usual (one part of 3.8 per cent sodium citrate and four parts of blood). This furnished citrated plasma and red blood cells. At the same time 5 ml. of blood was collected in a clean dry tube. The blood was allowed to clot and serum was separated from it. Five-tenths milliliter of 3.8 per cent

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Received for publication June 14, 1954.
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sodium citrate solution was added to 1.5 ml. of serum, and 0.5 ml. of centrifuged R.B.C. sediment (red blood cells of the same patient were used; the cells had been washed thrice with saline and centrifuged). In another tube 2 ml. of citrated plasma (approximately 1.5 ml. of plasma and 0.5 ml. of 3.8 per cent sodium citrate) and 0.5 ml. of R.B.C. sediment were mixed. Thus, all the factors in the two tubes were identical except that one had fibrinogen and the other had not. From each sample S.R. was determined in the warm and the cold chamber. A typical example out of ten samples tested is given in Table I.

TABLE I. VARIATION IN SEDIMENTATION RATES IN PLASMA AND SERUM IN RELATION TO TEMPERATURE

TIME (MIN.)	S.R. IN SERUM		S.R. IN PLASMA	
	99° F.	44° F.	99° F.	44° F.
10	12	90	10	90
20	27	143	22	140
30	50	157	45	163
40	90	158	60	169
50	112	159	80	169
60	134	160	120	170

The results indicate that irrespective of the presence or absence of fibrinogen, S.R. has been consistently higher in the colder temperature. As a matter of fact, earlier in our work we were struck by the observation that in many cases of hepatic cirrhosis, in spite of low blood fibrinogen levels, the S.R. was very high. The average fibrinogen level estimated in fifteen normal Indian individuals in this region was 318 mg. per cent, while in five patients with cirrhosis of the liver who had an average S.R. of 109 mm. the fibrinogen level was only 107 mg. per cent. The observation seemed rather significant and raised a doubt about fibrinogen alone being responsible for the accelerated S.R. irrespective of the temperature.

To determine the influence of different protein fractions, they were next separated. Albumin was separated by the technique described by Simons and Gentzkow.[3] Three milliliters of water and 5 ml. of saturated ammonium sulfate were added to 2 ml. of serum. The mixture was incubated for fifteen minutes at 37° C., and then filtered. To the filtrate 15 ml. of water and 5 ml. of 20 per cent trichloroacetic acid were added and it was then centrifuged. The supernatant fluid was thrown off and the remaining albumin precipitate was separated.

Salt fractionation of globulins was next carried out. Euglobulin was separated as described by Cole.[4] Fifteen milliliters of distilled water and 2 ml. of 1 per cent acetic acid were added to 2 ml. of serum. The precipitate was allowed to settle for one hour and was then separated from the supernatant fluid by centrifugation. The precipitate was euglobulin. To the supernatant fluid an equal amount of a saturated solution of ammonium sulfate was added. The resultant precipitate of pseudoglobulin and p-globulin was separated.

In one tube euglobulin, in the second pseudo- and p-globulin, and in the third albumin, pseudo- and p-globulin were dissolved in 0.5 ml. saline. Each

represented fractions separated from 2 ml. of serum. The effect of various fractions on the suspension stability of normal blood was studied.

A normal blood was collected as for Westergren's technique. Two milliliters of this blood was placed in each of the four test tubes. To the first euglobulin, to the second pseudo- and p-globulin, to the third pseudo-, p-globulin, and albumin, and to the fourth, the control, 0.5 ml. saline, was added. From each of the four tubes S.R. was determined in hot and in cold chambers. A typical result out of ten cases is given in Table II.

TABLE II. EFFECT OF EUGLOBULIN ON THE SEDIMENTATION RATE IN RELATION TO TEMPERATURE

TIME (MIN.)	S. R. OF THE PROTEIN DONOR		NORMAL BLOOD						CONTROL	
			WITH EUGLOBULIN		WITH P- AND PSEUDOGLOBULIN		WITH P- AND PSEUDOGLOBULIN AND ALBUMIN			
	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.
10	12	90	5	20	1	0	0	0	0	0
20	27	143	17	135	4	2	3	2	4	4
30	50	157	50	142	8	5	8	6	10	6
40	80	158	65	145	18	11	17	9	17	7
50	90	159	78	147	25	14	21	12	22	12
60	112	160	92	149	39	18	32	16	24	15

From Table II it is apparent that the accelerated S.R. in the cold temperature is completely simulated by the tube to which euglobulin was added. The readings in other tubes were very much like that of the control. It could, therefore, be concluded that the euglobulin fraction of protein is the essential factor which is responsible for the accelerated S.R. at the lower temperature.

Further experiments were then carried out to discover the influence of euglobulin (which was responsible for a high S.R. in cold) on the suspension stability of the blood which showed a high S.R. in a warm temperature. Euglobulin from a patient with liver cirrhosis was added to the blood of a tubercular patient. The technique used was the same as that described previously. One typical case out of five is presented in Table III.

TABLE III. EFFECT OF EUGLOBULIN FROM THE BLOOD OF PATIENT WITH CIRRHOSIS OF THE LIVER ON THE SEDIMENTATION RATE IN RELATION TO TEMPERATURE

TIME (MIN.)	ORIGINAL S.R. OF TUBERCULAR PATIENT		TUBERCULAR PATIENT WITH EUGLOBULIN FROM CIRRHOTIC		S.R. OF CIRRHOTIC	
	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.
10	4	2	12	50	55	145
20	12	2	15	120	65	156
30	27	3	20	127	90	157
40	35	4	80	133	120	160
50	54	5	94	142	135	162
60	58	8	102	145	146	162

It can be seen that the cases which originally showed accelerated S.R. in the warmer chamber now showed a decreased S.R., i.e., a higher rate in the cold than in the warm temperature.

To determine the influence of euglobulin obtained from individuals who showed a higher S.R. in the hot chamber, further experiments were performed. The euglobulin (from tubercular patients and healthy persons) was added to the normal blood and S.R. in hot and cold chambers was determined. Results are given in Tables IV and V.

TABLE IV. SEDIMENTATION RATE OF NORMAL BLOOD WITH EUGLOBULIN OF TUBERCULAR PATIENT

	CONTROL		NORMAL BLOOD WITH EUGLOBULIN		S.R. OF EUGLOBULIN DONOR	
TIME (MIN.)	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.
10	0	0	2	2	4	2
20	4	4	7	5	12	2
30	6	6	25	7	27	3
40	12	7	38	23	35	4
50	22	12	43	27	54	5
60	34	15	65	35	58	8

TABLE V. SEDIMENTATION RATE OF NORMAL BLOOD WITH ADDED EUGLOBULIN OF HEALTHY INDIVIDUAL

	CONTROL		NORMAL BLOOD WITH EUGLOBULIN		S.R. OF EUGLOBULIN DONOR	
TIME (MIN.)	99° F.	44° F.	99° F.	44° F.	99° F.	44° F.
10	0	0	3	0	3	2
20	4	4	9	8	9	2
30	8	6	16	10	12	5
40	17	7	22	12	15	7
50	22	12	32	12	17	11
60	34	15	45	16	18	15

It can be seen from the tables that the euglobulin donors had a higher S.R. in the hot chamber than in the cold chamber, and that addition of this euglobulin failed to reverse the higher S.R. at 99° F. in the bloods to which it was added. This result proved a qualitative difference between the euglobulins of the bloods which showed a high S.R. in the cold and those which did not. The qualitative change seems to be vested in the physical property of the euglobulin; further experiments are being carried out to determine its exact nature.

DISCUSSION

Euglobulin is probably a conjugated lipoprotein compound of pseudoglobulin and phospholipid, which has the same molecular weight as the other globulins, i.e., 170,000. It can be flocculated by carbon dioxide, dialysis, or dilution with water. Dutta,[5] who carried out fractionation of plasma proteins in normal individuals and cirrhotics, found that the globulin rise in cirrhotics was almost entirely due to an increase of euglobulin. He obtained values of 5.16 Gm. per cent as compared to the normal of 0.52 Gm. per cent.

From our investigations it becomes clear that an increased total euglobulin may cause the general increase of S.R. at any temperature, but that the accelerated S.R. in the cold is due to a qualitative change in the euglobulin fraction. It was seen that even minute amounts of this fraction succeeded in reversing the normal pattern of S.R.

It, therefore, appears that the common factors for flocculation reactions are increased euglobulin and decreased albumin. The causative agent of accelerated S.R. in the cold—the euglobulin—seems to undergo some qualitative change in hepatic disorder, as the same fraction separated from normal individuals or from those suffering from other diseases does not show this phenomenon.

SUMMARY

Experiments have been carried out to investigate the cause of greater S.R. at low temperature when the test is performed both in hot and cold chambers.

Various protein factions were separated by the salting-out technique and the influence of individual fractions was tested on the suspension stability of blood in hot and cold chambers.

The mechanism of this reaction seems to depend on a qualitative change of the euglobulin fraction of plasma proteins.

My thanks are due to Dr. R. J. Weingarten, Chief Physician and Principal Medical Officer of Bikaner State, for his encouragement and guidance throughout the work.

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LABORATORY METHODS

THE ESTIMATION OF DARSTINE, 1-(3-HYDROXY-5-METHYL-4-PHENYLHEXYL)-1-METHYLPIPERIDINIUM BROMIDE AND ITS TERTIARY ANALOG, 5-METHYL-4-PHENYL-1-(1-PIPERIDYL)-3-HEXANOL HYDROCHLORIDE, IN PLASMA AND URINE

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IT HAS been reported by McManus and associates[1] that Darstine,* 1-(3-hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide, is an anticholinergic agent with predominantly gastrointestinal affinity. Beyer and associates[2] have presented data on the physiologic economy of this agent. The methods described herein for the determination of Darstine and its corresponding tertiary analog were developed so that the distribution and renal fate of these drugs could be studied and compared.

PROCEDURE

Two methods will be presented. The first, a macromethod, is suitable for estimating Darstine as well as its tertiary counterpart in plasma and urine. The second, a microadaptation, is reliable only for determining the tertiary compound.

Reagents.—

1. 1 N Sodium hydroxide.
2. Ethylene dichloride: reagent grade that has been washed once with 1 N sodium hydroxide, once with 1 N hydrochloric acid and several times with distilled water.
3. Sodium chloride: reagent grade.
4. Methyl orange reagent: equal volumes of the following two solutions are mixed immediately before use:
   a. A 1:5 aqueous dilution of a saturated aqueous solution of methyl orange that has been washed with ethylene dichloride until the solvent layer is colorless.
   b. 0.5 M boric acid.
5. Alcoholic sulfuric acid: 2 ml. concentrated sulfuric acid is diluted to 100 ml. with absolute ethanol.
6. 1 N Hydrochloric acid.

Macroanalysis.—

1. One milliliter of the solution to be analyzed, 1 ml. of 1 N sodium hydroxide, and 20 ml. of ethylene dichloride are pipetted into a 60 ml. glass-stoppered bottle to which 1 Gm. of sodium chloride has also been added. The bottle then is shaken for fifteen minutes on a shaking apparatus.

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Received for publication June 24, 1954.

*Darstine is the trade-mark of Merck and Company, Inc., for its brand of 1-(3-hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide, the nonproprietary name of which is mepiperphenidol.

2. The solution is decanted into a test tube and centrifuged for 5 minutes, following which the aqueous layer is removed by aspiration.

3. The organic solvent layer is poured into a 60 ml. glass-stoppered bottle that contains 0.5 ml. of methyl orange reagent and is shaken for 5 minutes on a mechanical shaker. (Because the results are unreliable if the samples are allowed to stand in contact with the methyl orange for any length of time, it is desirable to carry no more than eight samples through this portion of the procedure at one time.)

4. Immediately upon the conclusion of the shaking period the sample is decanted into a test tube, centrifuged for 2 to 3 minutes, and the aqueous layer is removed by aspiration.

5. Ten milliliters of the ethylene dichloride layer is pipetted into a colorimeter tube that contains 1 ml. of alcoholic sulfuric acid solution. Within 5 to 10 minutes it is read colorimetrically at a wave length of approximately 525 mμ.

6. A reagent blank should be run through the above procedure with each group of samples assayed. These reagent blanks, which are highly reproducible, are used for setting the colorimeter. Plasma blanks, and urine blanks consisting of diluted urine, generally approximate the reagent blank.

Microanalysis.—

1. One milliliter of the solution to be analyzed, 1 ml. of 1 N sodium hydroxide, and 30 ml. of ethylene dichloride are pipetted into a 60 ml. glass-stoppered bottle to which 1 Gm. of sodium chloride has also been added. The bottle then is shaken for 15 minutes on a shaking apparatus.

2. The solution is decanted into a test tube and centrifuged for 5 minutes following which the aqueous layer is removed by aspiration.

3. The solvent layer is poured into a glass-stoppered bottle that contains 0.5 ml. of methyl orange reagent and is shaken for 5 minutes on a mechanical shaker.

4. Immediately upon the conclusion of the shaking period the sample is decanted into a test tube, centrifuged for 2 to 3 minutes, and the aqueous layer is removed by aspiration.

5. Twenty milliliters of the solvent layer is pipetted into a third glass-stoppered bottle that contains 1 ml. of 1 N hydrochloric acid and is shaken for 10 minutes.

6. The solution is poured into a test tube and then 0.3 to 0.4 ml. of the supernatant aqueous phase is pipetted into a microcuvette and its UV-extinction is determined at a wave length of 508 mμ, using a microattachment to the Beckman spectrophotometer.

7. A reagent blank with water substituted for plasma or urine should be run with each group of determinations. The extinction coefficient of this blank when compared with 1 N HCl should not be greater than 0.025. If a greater value is obtained, it is probably an indication that the glassware is not entirely free from detergents, acids, or alkalies. The difference between plasma blanks and the reagent blank should not be greater than 0.075.

RESULTS

A straight-line standard curve is obtained when solutions of Darstine ranging in concentration from 0.5 to 7.0 mg. per 100 ml. are carried through the macroprocedure. Fig. 1 illustrates the difference between an aqueous curve and one prepared in plasma. Since these curves are each completely reproducible, we have found it most convenient to prepare both for use in calculating the concentration of Darstine in unknown samples.

There is a consistent 10 per cent difference between the two curves, which makes it possible to use an aqueous standard for calculating plasma concentrations should a plasma standard be unavailable. This is illustrated in Table I. To obtain these data standard solutions of Darstine in the concentrations listed in the first column were prepared. These were carried through the macro-

procedure and the results were calculated by referring to the aqueous curve. The second column shows the values thus obtained, while the third and fourth columns give the corrected concentrations and the per cent recovery.

A reproducible straight-line curve is also obtained when standard solutions of 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol hydrochloride are carried through this procedure. In this instance, the plasma and aqueous curves are similar, which makes it possible to use an aqueous standard in calculating plasma unknowns. This is illustrated in Table II, which presents plasma recovery data obtained when an aqueous standard was used.

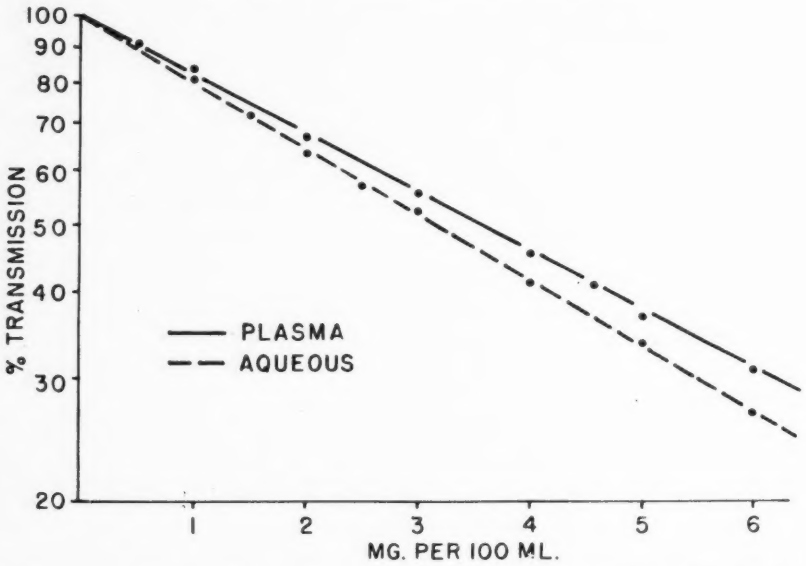

Fig. 1.—Comparison of standard curves of Darstine prepared in plasma and distilled water.

The micromethod is not reliable for Darstine determinations. Although we have occasionally obtained reproducible results by this modification, more frequently erratic values are to be expected. Fortunately, in experimental work the macromethod for Darstine is generally sensitive enough, since it is possible to run the assay on 2 to 5 ml. aliquots of plasma. Increasing the initial volume in this way does not alter plasma recoveries if sufficient sodium chloride to saturate the aqueous phase is also added. However, when the corresponding piperidine tertiary analog of Darstine is administered to laboratory animals the resulting plasma and urine concentrations of the drug are so low that it is necessary to use the microtechnic for their estimation.

A reproducible, straight-line standard curve is obtained when standard solutions of the tertiary analog of Darstine ranging in concentration from 0.3 to 1.0 mg. per 100 ml. are carried through the microprocedure. Here again the standard curve prepared in plasma may be superimposed on the aqueous curve, making it possible to use the latter in calculating plasma values. This is illustrated in Table III. To obtain these data plasma dilutions of the drug in the concentrations listed in the first column were prepared.

TABLE I. PLASMA RECOVERIES OF DARSTINE

DRUG ADDED (MG./100 ML.)	VALUE FROM AQUEOUS CURVE	(× 10/9) MG./100 ML.	% RECOVERY
0.5	0.45	0.50	100.0
1.0	0.80	0.89	89.0
1.5	1.30	1.44	96.0
2.0	1.825	2.03	101.5
2.0	1.85	2.06	103.0
2.5	2.30	2.56	102.4
3.0	2.65	2.94	98.0
4.0	3.60	4.00	100.0
4.0	3.55	3.94	98.5
4.5	4.05	4.50	100.0
5.0	4.47	4.97	99.4
5.0	4.40	4.89	97.8
6.0	5.10	5.67	94.5
6.0	5.52	6.13	102.2

TABLE II. MACROMETHOD: PLASMA RECOVERIES OF 5-METHYL-4-PHENYL-1-(1-PIPERIDYL)-3-HEXANOL HYDROCHLORIDE

DRUG ADDED (MG./100 ML.)	DRUG RECOVERED (MG./100 ML.)	% RECOVERY
3.0	3.15	105.0
5.0	5.15	102.0
6.0	5.65	94.2
1.0	0.90	90.0
3.0	2.90	96.7
4.0	3.9	97.5
6.0	5.65	94.2
1.0	1.0	100.0
2.0	2.1	105.0
3.0	2.9	96.7
4.0	3.8	95.0
5.0	5.1	102.0
6.0	5.95	99.2

TABLE III. MICROMETHOD: PLASMA RECOVERIES OF 5-METHYL-4-PHENYL-1-(1-PIPERIDYL)-3-HEXANOL HYDROCHLORIDE

DRUG ADDED (MG./100 ML.)	DRUG RECOVERED (MG./100 ML.)	% RECOVERY
0.5	0.49	98.0
0.8	0.78	97.5
0.3	0.30	100.0
0.9	0.89	98.9
0.7	0.69	98.5
0.5	0.48	96.0
0.3	0.28	93.3
1.0	0.97	97.0
0.8	0.78	97.5
0.6	0.59	98.3
0.4	0.38	95.0

These were analyzed by the micromethod. The concentrations and per cent recovery obtained when an aqueous curve is used in the calculations are presented in the second and third columns.

In studying the fate of Darstine it was desirable to have a method for differentiating between it, its tertiary analog, and N′-methylpiperidinium bromide (which can also be estimated by these methods), to determine whether

Darstine is converted to either of the other compounds in the course of its metabolism. Fortunately, Darstine, unlike the others, is extremely insoluble in chloroform, which makes the following differentiation possible.

Two volumes of a solution, containing no more than 3 μg per milliliter of total drug and two volumes of Clark and Lubs buffer, pH 9,[3] are pipetted into a glass-stoppered bottle. Twenty volumes of washed chloroform* are added and the bottle is shaken on a mechanical shaking apparatus for five minutes. The solvent layer is removed by aspiration. At this pH both 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol hydrochloride and N′-methylpiperidinium bromide are extracted while Darstine remains in the aqueous phase. When the extraction is carried out with McIlvaine's buffer, pH 5,[4] substituted for the pH 9 buffer, 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol hydrochloride is extracted and both Darstine and N′-methylpiperidinium bromide remain in the aqueous layer.

When samples of plasma and urine collected following the administration of Darstine are subjected to the above treatment, in no instance has there been evidence of N′-methylpiperidinium bromide or 5-methyl-4-phenyl-1-(1-piperidyl)-3-hexanol hydrochloride. All the drug found seems to be Darstine or a similar compound that is likewise insoluble in chloroform and which cannot be differentiated from it by the methods described herein.

SUMMARY

A method for the estimation of Darstine and its corresponding piperidine tertiary analog in plasma and urine has been described. The method depends on the fact that the drugs can be extracted into ethylene dichloride from an alkaline, aqueous medium. The solvent layer is shaken then with an aqueous solution of methyl orange and the colored salt formed at the interface, which is soluble in ethylene dichloride, is acidified and read colorimetrically.

In the microadaptation that is reliable only for estimating the tertiary compound, the colored salt is re-extracted from the solvent into dilute acid and its absorbency is determined.

A method for differentiating between the two drugs has been described also. This depends on the fact that chloroform extracts the tertiary compound but not Darstine from an aqueous solution.

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*A reagent grade of chloroform is purified by shaking successively with 1 N sodium hydroxide and 1 N hydrochloric acid, and following with several washes with water.

A NEW METHOD FOR ANALYSIS OF PLASMA FIBRINOGEN, UTILIZING A CATIONIC DETERGENT

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IN A previously reported investigation, it was demonstrated that utilization of a reaction of serum proteins with cationic detergents permitted quantitative fractionation of albumin, the beta-gamma fraction and the alpha-globulin component of human serum.[1] In this study, plasma fibrinogen was also observed to react with cationic detergents to form a turbid suspension which could be measured by photometric methods. However, certain characteristics of the fibrinogen-detergent reaction in plasma prohibited accurate assay by the method previously described.

The present investigation describes a technique for cationic detergent analysis of the plasma fibrinogen concentration. This method is compared with a clotting technique conventionally used for determination of plasma fibrinogen concentration.

EXPERIMENTAL METHOD

When plasma rather than serum is utilized in the detergent assay of its component protein fractions, the fibrinogen of plasma is observed to precipitate in the alpha-globulin partition. If fibrinogen is present in a low concentration, little error is introduced; a high concentration produces an appreciable error in the alpha-globulin determination. It might be considered that simultaneous analysis of serum and plasma from the same donor would afford a basis for calculating fibrinogen concentration by a determination of the difference of globulin concentration in the two samples. Because a hundredfold dilution of serum is required to make a fractional analysis of serum proteins,[1] a similar dilution of plasma causes sufficient decrease of the fibrinogen concentration to prohibit an accurate analysis by a differential calculation of the serum and plasma values of the "alpha globulin fraction."

A separation of the other plasma components from fibrinogen by precipitation with a 28.5 per cent saturation of ammonium sulfate eliminates the interfering action of these components. The precipitated fibrinogen, redissolved in a 1 per cent sodium chloride solution, can then be analyzed in a menstruum, relatively free of other plasma components. When the pH of the solution containing fibrinogen is below pH 6.0, any overlapping reaction of other globulins coprecipitating with fibrinogen is avoided. The addition of 0.4 M sodium chloride to this buffer solution increases the precipitation of fibrinogen with cationic detergents without simultaneously increasing detergent precipitation of other globulins which might be present.

Preparation of Reagents.—Fibrinogen: Through the generosity of Dr. Walter Seegers, a sample of human fibrinogen was made available. This material is 90 per cent clottable and, after removal of the clot, the remaining solution is found to contain only 5 per cent residual protein material. Fibrinogen is weighed and diluted in sufficient 1 per cent NaCl solution to make concentration of 0.60-0.80 Gm./100 ml. Such a preparation of purified fibrinogen is slowly soluble in 1 per cent NaCl and forms a clear solution which is stable for three to four hours. After this time it spontaneously precipitates and can no longer

Research carried out under a grant for the study of rheumatic fever from the Masonic Foundation for Medical Research and Human Welfare.
Received for publication July 16, 1954.
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be used. The prepared solution of fibrinogen is assayed for total nitrogen by the Kjeldahl method. The value obtained, multiplied by the factor 6.25, is used as the fibrinogen concentration of the particular solution employed.

Phosphate buffer: A 0.1 M Na_2HPO_4 solution is made. To this sufficient NaCl is added to make the solution 0.4 M with respect to NaCl. A 0.1 M NaH_2PO_4 solution is prepared. Sodium chloride is similiarly added to make a 0.4 M concentration of NaCl. These two solutions are then mixed together in suitable proportions so that a pH of 5.1 results.

Cationic detergent solution: Octadecyl dimethyl benzyl ammonium chloride (Octab) is dissolved in distilled water to make a 0.1 per cent concentration.

Ammonium sulfate solution: Sufficient $(NH_4)_2SO_4$ is added to distilled water to make a saturated solution.

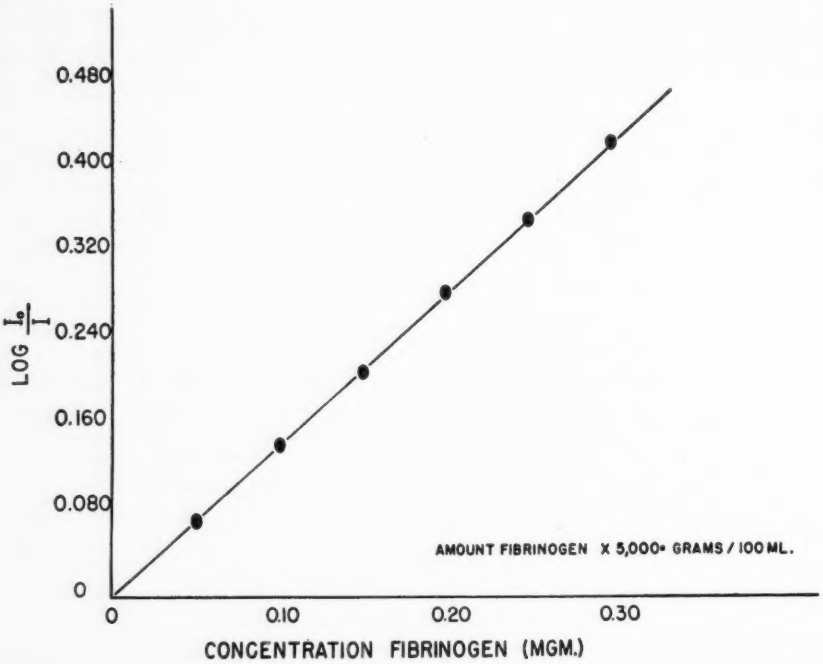

Fig. 1.—Concentration curve for fibrinogen. The concentration is plotted against the optical density obtained after reaction with the cationic detergent.

Assay of Fibrinogen: One milliliter of oxalated plasma is introduced from a 1.0 ml. volumetric pipette into a graduated, conical centrifuge tube. To this 4.0 ml. of 1 per cent NaCl solution are added; 2.0 ml. of saturated ammonium sulfate are then pipetted into the centrifuge tube and mixed thoroughly with the diluted plasma. After five to ten minutes, the precipitated protein is flocculated into visible clumps. The tube is then centrifuged at 1,500 r.p.m. for ten minutes. The fibrinogen collects at the bottom as a pellet and the supernatant solution can be removed by gentle decantation. Occasionally, flecks of precipitate rise to the surface of the liquid. If the tube is slowly tilted to allow the supernatant solution to slowly run out, these flecks adhere to the glass wall. After inversion of the centrifuge tube, remaining drops of supernatant solution are clotted from the rim with a cotton gauze pledget.

A NaCl solution, 1 per cent, is added to the graduated centrifuge to a volume of 5.0 ml. By means of a wooden applicator stick, the precipitate at the bottom of the tube and on the side wall is mixed, and redissolved to form a clear solution.

One-tenth milliliter of this solution (measured in a 0.2 ml. pipette) is removed from the centrifuge tube and placed in a colorimeter cuvette. To this is added 8.9 ml. of the

phosphate—NaCl buffer solution. One milliliter of 0.1 per cent Octab is then pipetted into this mixture and immediately mixed by inverting the tube several times.

The detergent fibrinogen solution quickly becomes opalescent and maximal density is formed within ten to fifteen minutes. After twenty-five minutes, the precipitate slowly coalesces to form larger particles and a decrease in density results. Therefore, at approximately fifteen minutes after addition of the Octab solution, the optical density of the solution is determined. For this, a Lumetron Colorimeter containing a 440 filter is used. This colorimeter is best adapted for determination of turbidity because of physical principles previously described.[1]

Preparation of the Standard Curve: The solution of purified human fibrinogen of known concentration is suitably diluted in 1 per cent NaCl so that each milliliter contains 2.0 mg. Aliquots of this sample are then added to different tubes so that a range of fibrinogen concentration between 0.05 and 3.0 mg. results. To each of these samples, phosphate buffer and detergent are added to a volume of 10.0 ml. The optical density is determined. These values are then plotted against the Kjeldahl determined fibrinogen concentration and a standard curve such as is shown in Fig. 1 is constructed.

EXPERIMENTAL

Known concentrations of the fibrinogen solution are added to sera obtained from patients with various pathologic lesions. In most instances these sera contain increased concentrations of the alpha-globulins or the beta-gamma fraction. The serum fibrinogen mixture is then precipitated with 28.5 per cent ammonium sulfate, and analysis of fibrinogen is made as described previously.

The data obtained by this procedure are shown in Table I. It will be observed that recovery of fibrinogen varies between 90 and 113 per cent. The

TABLE I. RECOVERY EXPERIMENTS IN WHICH KNOWN CONCENTRATIONS OF HUMAN FIBRINOGEN ARE ADDED TO ABNORMAL SERA AND THEN ASSAYED BY THE DETERGENT TECHNIQUE

PATIENT	ALBUMIN (GM.)	ALPHA GLOBULIN (GM.)	BETA AND GAMMA GLOBULIN (GM.)	THEORECTICAL YIELD FIBRINOGEN (GM.)	ACTUAL YIELD FIBRINOGEN (GM.)	PER CENT RECOVERY*
1	4.55	1.3	1.55	0.410	0.450	110
2	4.1	1.35	1.45	0.410	0.425	103
3	4.0	1.25	0.90	0.410	0.425	103
4	4.35	1.3	1.1	0.410	0.425	103
5	2.2	1.9	1.2	0.410	0.415	101
6	3.4	2.25	1.25	0.410	0.450	110
7	3.25	2.0	2.0	0.410	0.450	110
8	3.8	1.5	1.1	0.410	0.450	110
9	1.85	2.6	1.0	0.410	0.425	103
10	3.9	1.2	1.05	0.410	0.450	110
11	3.6	1.9	1.2	0.840	0.875	104
12	4.3	1.6	2.7	0.840	0.865	103
13	2.2	2.5	1.0	0.840	0.865	103
14	3.9	1.3	1.4	0.840	0.840	100
15	4.2	1.1	1.0	0.840	0.775	92
16	4.7	0.9	2.6	0.840	0.850	101
17	3.2	2.2	2.5	0.840	0.900	107
18	2.6	2.3	2.1	0.840	0.950	113
19	2.2	1.7	1.4	0.840	0.800	95
20	3.2	2.4	2.9	0.910	0.865	103
				0.091	0.082	90
21	2.4	2.2	3.1	0.910	0.890	98
				0.091	0.100	110
22	2.9	2.0	3.2	0.910	0.850	93
				0.091	0.100	110
23	2.2	2.85	1.95	0.740	0.775	105
				0.074	0.082	111

*Mean 103.7 per cent ± 6.1 per cent.

TABLE II. COMPARISON OF THE DETERGENT METHOD FOR ANALYSIS OF PLASMA FIBRINOGEN LEVEL WITH THE CLOT PRECIPITATION METHOD

PATIENT	FIBRINOGEN BY DETERGENT METHOD	FIBRINOGEN BY CLOT METHOD	DETERGENT YIELD COMPARED TO CLOT METHOD (PER CENT)
1	0.315	0.260	121
2	0.565	0.450	125
3	0.630	0.550	114
4	0.760	0.585	130
5	0.735	0.550	134
6	0.620	0.463	133
7	0.465	0.400	116
8	1.040	0.860	121
9	0.750	0.670	112
10	1.010	0.875	115
11	0.610	0.505	120
12	1.150	0.980	117

mean recovery is 103.7 ± 6.1 per cent. Whether the concentration of fibrinogen added to the serum samples is large or small, the analytic results show comparable recovery results. An excess of globulins, either of the alpha or of the beta-gamma fractions does not influence the analytic data for fibrinogen.

Analysis of fibrinogen in plasma was made by a clotting technique in which fibrin is formed after addition of $CaCl_2$ solution to plasma.[2] The fibrin is then removed on a glass applicator and analyzed by determination of the nitrogen by the Kjeldahl technique. This method was compared with the detergent analytic method described previously. The values obtained from the same plasma from different patients are shown in Table II. It will be observed that consistently higher values are found by the latter method. This increment significantly exceeds the error found in the experiments wherein known concentrations of fibrinogen are added to serum (Table I).

In order to study this discrepancy, fibrin clots were lysed in serum by means of streptokinase digestion. The lysed, nonclottable fibrin was then precipitated with 28.5 per cent saturation of ammonium sulfate and assayed. Identical concentrations of "fibrinogen" are found in the lysed sample as are contained in the unclotted preparation of fibrinogen added to the same serum.

An example of spontaneous clot lysis was studied in a child with hemolytic disease of the newborn. In this instance, the blood clotted normally, but at 37° C. the clotted sample underwent rapid lysis. Addition of thrombin to the lysed sample failed to reproduce a fibrin clot, but detergent analysis of this serum produced "fibrinogen" values identical with the corresponding plasma fibrinogen level. Thus it appears that spontaneous lysis of fibrin and streptokinase lysis of fibrin alter the molecular structure sufficiently to prevent any reconversion by thrombin, but the molecular structure is sufficiently intact so that precipitation with $(NH_4)_2SO_4$ and reaction with the detergent is unaffected by the lytic agent.

It is possible, therefore, that detergent analysis of the plasma fibrinogen may include not only the clottable fraction, but also any nonclottable or lysed fibrin component. This may be a partial explanation of the discrepancy depicted in the data shown in Fig. 1.

DISCUSSION

By precipitating fibrinogen from plasma with 28.5 per cent ammonium sulfate, the major portion of the other plasma proteins can be separated from the fibrinogen fraction. On redissolving the fibrinogen in sodium chloride solution, it can be analyzed by the cationic detergent method. Any other plasma proteins adherent to the walls of the test tube or coprecipitating with fibrinogen are not reactive with detergent provided the buffer has a sufficiently low pH. Moreover, addition of 0.4 M NaCl to a phosphate buffer solution of pH 5.1, increases the fibrinogen reaction with a cationic detergent without causing a similar increase of the alpha globulins. Thus, in sera with high concentrations of globulins, i.e., gamma globulin, which may partially precipitate with fibrinogen in a 28.5 per cent ammonium sulfate solution, the low pH and high NaCl concentration permit a selective analysis of fibrinogen.

Known amounts of fibrinogen were added to abnormal sera in concentrations between 90 to 900 mg./100 ml. and the mixtures were assayed by the detergent analytic method. These samples could be analyzed with 103.7 ± 6.1 per cent accuracy.

The property of lysed fibrin to precipitate with 28.5 per cent ammonium sulfate and to react with a cationic detergent in the same manner as unaltered fibrinogen may explain the higher values of plasma fibrinogen found in the detergent analysis technique than were obtained with the clotting technique.

SUMMARY

A new technique for analysis of plasma fibrinogen is described. This method utilizes the property of fibrinogen to react with a cationic detergent, octadecyl dimethyl benzyl ammonium chloride, at a low pH and a high sodium chloride concentration.

This method, while permitting accurate recovery of fibrinogen added to serum, produced higher values when plasma fibrinogen analysis by detergent was compared with a clotting method for fibrinogen assay. A possible explanation for this discrepancy may depend on the fact that the cationic detergent reacts with lysed fibrin in the same manner as with fibrinogen.

Grateful acknowledgment is made to Margaret Cushman, M.A., Rochester, New York, for her assistance in the laboratory determinations.

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Erratum

In the article by Rose and Rawlings entitled "The Local Anesthetic Action of Certain Amides," which appeared in the October, 1954, issue of the JOURNAL, pages 571 to 581, the first part of line 3 of paragraph 3 should read as follows: "median lethal dose (LD_{50} ± Standard Error)."

PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twenty-Seventh Annual Meeting

Chicago, Ill., Oct. 29 and 30, 1954

ABSTRACTS

104. EXPERIENCE WITH 1,4-DIMETHANESULFONOXYBUTANE (MYLERAN) IN THE TREATMENT OF CHRONIC MYELOCYTIC LEUKEMIA

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Six patients with chronic myelocytic leukemia have been treated with 1,4-dimethanesulfonoxybutane (Myleran). The dosage schedule in each case has been 4 to 6 mg. per day. The patients have been maintained on 2 to 6 mg. per day, with variable rest periods, depending upon each patient's individual response. Careful hematological and clinical controls were used.

In every instance there was initial clinical and subjective improvement, which usually became apparent within one to seven days after institution of therapy. There was also constant hematological improvement, although one to four weeks elapsed before the hematological alterations became maximal. Improvement was in each case characterized by a fall in the total leukocyte count, restoration of absolute numbers of mature neutrophils, reduction in the severity of anemia, and maintenance of platelet values at or above normal in all but one case. Clinical improvement was very striking. In every case, the patient reported dramatic improvement subjectively. This was followed shortly by reduction of the degree of hepatosplenomegaly and subsidence of symptoms of hypermetabolism. In one case, characterized by severe symptoms due to infiltration of the kidneys, there was prompt regression following the institution of therapy.

No troublesome gastrointestinal symptoms, such as are encountered in patients being treated with external irradiation or with radiomimetic drugs, were reported. One patient has developed moderate marrow hypoplasia, but there is now evidence of spontaneous marrow regeneration. The most alarming occurrence in this series is the fact that two of the six patients have developed acute myeloblastic transformations and promptly succumbed. Admittedly, it is hazardous to draw sweeping conclusions from so few cases, but we feel that there is a need to further evaluate the possibility that this agent may in some instances potentiate the virulence of chronic myelocytic leukemia. Nevertheless, this chemotherapeutic agent offers another effective method of palliation in patients with chronic myelocytic leukemia.

105. THE EFFECT OF THYROTROPIN AND DESICCATED THYROID ON HYPOTHYROIDISM WITH GOITER

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(INTRODUCED BY WALTER H. PRITCHARD, M.D.)

There has been increasing recognition of the apparent paradox of hypothyroidism with concomitant thyroid overactivity and goiter formation. Although less than thirty cases have been reported, seven such patients have been encountered at this institution within two years. In addition to customary tests of thyroid function, the response to thyrotropin and to administration of desiccated thyroid has been evaluated.

With one exception the patients were children, and the degree of the hypothyroidism varied considerably as measured by the clinical state, basal metabolism rate, serum cholesterol level, and abnormalities in bone development. There was no history of previous ingestion of antithyroid drugs or goitrogens. Although the patients were not related to each other, one had a goitrous sister and another an infant sibling with hypothyroidism.

In the four cases tested prior to thyroid therapy, I^{131} uptake was elevated and did not respond to exogenous thyrotropin. The PBI was low in two, elevated in one, and normal in the other, the last being the only instance in which this index responded to thyrotropin. Iodide administration to the child with the elevated PBI was associated with further enlargement of the goiter without improvement of the hypothyroidism. Desiccated thyroid therapy had the following effects: decrease in goiter size, change in PBI toward normal regardless of its initial level, and decline in I^{131} uptake. As the uptake and PBI became more normal, responsiveness to thyrotropin administration developed. It is evident that the presence of goiter, elevated I^{131} uptake, and elevated PBI are not necessarily indicative of hyperthyroidism.

These studies provide further evidence that this syndrome is a manifestation of an intrinsic defect in the ability of the thyroid gland to synthesize thyroid hormone properly. This defect may occur either before or after the incorporation of iodine into an organic molecule, and it may be relative rather than absolute. The number of cases observed suggests that this disorder may be more common than has been thought previously.

106. MECHANISMS CONTROLLING CATION CONCENTRATIONS IN THE HUMAN CELL: EVIDENCE FROM THE EFFECT OF IODOACETATE ON SODIUM AND POTASSIUM EXCHANGE RATES OF THE HUMAN ERYTHROCYTE

W. D. LOVE, M.D. (BY INVITATION), J. A. CRONVICH, B.S. (BY INVITATION), AND G. E. BURCH, M.D., NEW ORLEANS, LA.

The mechanisms which control the concentration of sodium and potassium within the cells of the body are of clinical importance because of the discovery that intracellular electrolyte levels become abnormal during the course of a variety of illnesses. Previous studies of human red cells (R.B.C.) in vitro have led to the concept that the high potassium and low sodium concentrations in this cell are maintained, in part at least, by glycolysis-dependent active processes which produce inward transport of K and expulsion of Na. The measurements of rates of exchange of Na and K between plasma and R.B.C. which

form the basis of these previous interpretations have been made on cells incubated in artificial media, using methods which permitted only a single determination of exchange rate. In the present study, the time course of the changes in the rates of intake and output of Na and K by R.B.C. was determined in cells with and without Na-mono-iodoacetate (10^{-3} M.) added to inhibit glycolysis. R.B.C. were incubated under simulated physiologic conditions in heparinized plasma. K^{42}, Rb^{86}, and Na^{22} were used as tracers, Rb^{86} having been shown to be an adequate tracer for potassium in R.B.C.

During seven hours of incubation no significant net exchanges occurred in cells without added iodoacetate. In iodoacetate-treated cells the rate of net loss of potassium was 50 per cent greater than the rate of net gain of sodium (1.80 meq. K/L. R.B.C./hr. and 1.18 meq. Na/L. R.B.C./hr.). Determination of exchange rates showed that the increase in R.B.C. sodium was caused by a decrease of approximately one-third in the R.B.C. sodium output rate, whereas previous reports have indicated complete cessation of sodium output. Potassium intake was decreased to one-third of the normal value by the fourth hour after the addition of iodoacetate, while output showed an early and temporary increase to approximately twice the control value.

These results indicate the presence and magnitude of glycolysis-dependent processes controlling the intake and output of Na and K by R.B.C. Similar mechanisms may mediate the changes in cation content which occur in other cells during disease.

107. BONE MARROW STUDIES IN CARCINOMA OF THE PROSTATE

John Louis, M.D. (By Invitation), William R. Best, M.D., and Louis R. Limarzi, M.D., Chicago, Ill.

Specific marrow sites have been reported as superior for the finding of metastatic malignant cells in patients with carcinoma of the prostate. To confirm such findings, simultaneous marrow aspirations from the sternum, spinous process, and ilium were performed in 34 patients with prostatic carcinoma. Stony hard prostatic masses were present in all cases. Needle or operative biopsies were positive for adenocarcinoma in 18, questionable in 6, and negative in 4. Duration of symptoms prior to marrow aspiration was three weeks to fifteen years. Twenty-eight patients received either surgery, hormone therapy, or both, prior to marrow aspiration. The acid phosphatase was elevated in 8 patients. Roentgenographic evidence for metastatic bone lesions was present in 9 patients: 4 in the pelvis, 2 in the long bones, 1 in the spine, 1 in the first two sites, and 1 in all three. Blood hemoglobin less than 12 Gm. was noted in 11.

Bone marrows were considered to have metastasis if groups of tumor cells were found. Such cells were seen in 4 patients. Two patients had ungrouped cells which were suspected of being tumor cells. In 1 patient, all three marrow sites were abnormal, in 2 both the spinous process and ilium, in 1 the sternum and spinous process, in 1 only the sternum, and in the other only the spinous process. Among these 6 patients rediolucent bone lesions were present in 4, absent in 1, and questionable in the other. Acid phosphatase was elevated in 3. Prostatic biopsy was positive in 4 and questionable in 2. One patient had anemia. All 6 patients had received continuous hormone therapy for six months to three years. Transurethral resections were performed in three. In addition, one received a bilateral orchidectomy and adrenalectomy. The bone marrow showed an increased granulocytic:erythrocytic ratio and a left shift in the granulocytic elements in 5. Megakaryopoiesis was increased in 1. The remaining 28 patients had normal or nonspecific marrow patterns.

These preliminary findings re-emphasize the need for study of multiple marrow sites in the search for metastasis. They do not conclusively establish the superiority of any one site.

108. THE EFFECT OF DIODRAST ON THE CLOTTING TIME IN HEMOPHILIC PATIENTS

JOHN LOUIS, M.D., CHICAGO, ILL.

(INTRODUCED BY LOUIS R. LIMARZI, M.D.)

Diodrast (iodopyracet) has been reported to be effective in reducing the coagulation time and stopping hemorrhage in hemophilic patients (M. J. Australia **2**: 149-152, 1951). The effects of Diodrast on the coagulation time and hemorrhagic phenomena were studied in four patients. Age range was from 20 to 30 years. Diagnosis was established by clinical features in 4 and by low prothrombin consumption in 3. The Lee-White method was used to measure coagulation time. Intravenous doses of 20 ml. Diodrast (8.1 Gm. 3,5-diiodo-4-pyridone-N-acetic acid diethanolamine; 1.9 Gm. 3,5-diiodo-4-pyridone-N-acetic acid diethylamine) were given.

In one patient massive bleeding ceased after two doses of Diodrast given twelve hours apart. In the second patient, coagulation time was reduced from 4 hours to 30 minutes after one dose. However, bleeding did not stop. The other two patients were not bleeding. Coagulation time was reduced from 1 hour and 45 minutes to 40 minutes in one and from 5 hours and 45 minutes to 3 hours and 30 minutes in the other. In vitro studies of the blood in one patient showed prolongation of the clotting time by Diodrast. Further study will be required to determine the mechanism of action and clinical value of this drug in hemophilia.

109. TREATMENT OF CHRONIC GRANULOCYTIC LEUKEMIA WITH MYLERAN (1,4-DIMETHANESULFONOXYBUTANE)

JOHN LOUIS, M.D. (BY INVITATION), WILLIAM R. BEST, M.D., AND LOUIS R. LIMARZI, M.D., CHICAGO, ILL.

Favorable results using Myleran in the treatment of chronic granulocytic leukemia have recently been reported. Eleven patients having chronic granulocytic leukemia were therefore treated with Myleran. Seven were women. Ages ranged from 2 to 55 years. Duration of symptoms prior to administration of Myleran was three weeks to four years. All but two had been previously treated with other forms of therapy. Initial white counts varied from 22,300 to 170,000 per cubic millimeter. The initial dose of Myleran was 6 mg./d. in all but three; two received 4 mg./d. and one received 1 mg./d. The daily dose was reduced as the white count decreased. White counts became normal (i.e., less than 10,000) in all patients within one to twelve weeks, and required total doses of 70 to 476 mg. In general, hematological improvement was accompanied by increased feeling of well-being. Subsequent maintenance dosage varied from 1 to 4 mg./d. Two patients remained in peripheral blood remission for three weeks and one patient for four weeks, even though the agent had been discontinued. Leukopenia (white counts less than 4,500) occurred in three patients. As the white blood count decreased, the hemoglobin rose in all except one patient. Six patients were anemic prior to therapy. Anemia was no longer present after a peripheral blood remission occurred. In the four patients with moderate to marked splenomegaly, the spleens were no longer pal-

pable in two and markedly reduced in size in the remainder following peripheral remission. Complete marrow remissions were not observed to date. One patient died from a pseudomonas septicemia associated with marked leukopenia.

110. EFFECTS UPON METABOLISM AND UPON PITUITARY-ADRENAL FUNCTION IN MAN OF GLYCYRRHIZINIC ACID, THE ELECTROLYTE-ACTIVE PRINCIPLE OF LICORICE

LAWRENCE H. LOUIS, SC.D. (BY INVITATION), AND JEROME W. CONN, M.D., ANN ARBOR, MICH.

Dutch and English investigators have reported that crude licorice extract produces in man profound retention of sodium, chloride, and water as well as loss of potassium. The last Netherlands report (Pelser, Metabolism, 1953) indicates that in this respect glycyrrhizinic acid is the active principle of licorice. Six grams of the chemically pure material was available and was tested in an Addisonian patient during a two-day period.

Early in 1952 one of us (L. H. L.) succeeded in isolating from commercial licorice extract a 10 per cent yield by weight of chemically pure crystalline ammonium glycyrrhizinate. Identification has been established by chemical analysis. Since then a total of 366 Gm. of the pure compound has been administered to seven normal people, two adrenogenital and one Cushing's syndrome patient to determine its over-all metabolic and physiologic effects. The chemical configuration of ammonium glycyrrhizinate suggests that activity is due to similarity in structure to adrenal steroids or possibly to metabolic transformation to an active steroid. If so, the possibility existed that it would depress the pituitary-adrenal system. The other purpose was to determine its over-all metabolic effects with respect to both electrolyte and organic metabolism.

The results indicate the following: (a) Glycyrrhizinic acid is the substance in licorice which is responsible for the metabolic effects. (b) Great retention of sodium, chloride, and water constitute the major metabolic effects. Potassium loss occurs but is small. (c) Concentrations of sodium and chloride in thermal sweat are lowered sharply. (d) Protein, carbohydrate, and uric acid metabolism are not affected significantly nor are circulating eosinophils. (e) Inhibition of the pituitary-adrenal system is indicated by significant decreases of urinary 17-ketosteroids. (f) The total metabolic and physiologic effects indicate that ammonium glycyrrhizinate exerts activities which characteristically are produced by some adrenal cortical steroids.

111. EXTRACELLULAR FACTORS IN THE PATHOGENESIS OF CONGESTIVE HEART FAILURE: EFFECT OF PHYSICAL ACTIVITY ON PLASMA ELECTROLYTES IN PATIENTS WITH CONGESTIVE HEART FAILURE

IVAN J. MADER, M.D. (BY INVITATION), ALBERT J. BOYLE, M.D., PH.D., (BY INVITATION), AND LLOYD T. ISERI, M.D., DETROIT, MICH.

Previous studies suggest the possibility that when cardiac failure occurs there is increased intracellular osmolarity leading to extrusion of electrolytes out of and migration of water into the cells. Cellular demand for water presumably accounts for renal retention of water through ADH activity. The necessity for the extracellular compartment to increase its osmolarity in keeping with the increase within the cells may possibly account for renal retention

of sodium through adrenal cortical activity. Previously, plasma sodium levels in untreated cardiac patients in congestive heart failure were found to be elevated.

The present studies were undertaken to study the effect of acute and chronic physical activity on plasma sodium, potassium, chloride, and CO_2 levels in 17 cardiac patients who were, at the time of study, progressively going into congestive failure. Duplicate analyses were made in each case. Circulation time, venous pressure, and body weight were obtained serially.

During the period of progressive failure, a 4 Gm. NaCl load followed an hour later by acute physical stress (Master's two-step test or level walk) resulted in a significant rise in plasma sodium concentrations in 8 out of 10 patients. Of two patients showing no rise in plasma sodium, one had a markedly elevated level at the onset precluding a further rise. The other patient was not subjected to sufficient circulatory stress as indicated by the absence of dyspnea and a rise in venous pressure. The sodium load alone failed to increase the plasma sodium level. Normal subjects undergoing strenuous exercise also demonstrated a rise in plasma sodium. After compensation, comparable physical stress failed to produce any rise in plasma sodium in 4 out of 4 cardiac patients.

Six cardiac patients with progressive failure showed a rise in plasma sodium averaging 4.7 meq./L. between the early morning fasting and the late evening period after a day of unrestricted activity and diet. One diabetic patient with K-W syndrome showed a fall in plasma sodium explainable by the development of lipemic sera.

The findings will be discussed with respect to pathogenesis of congestive failure.

112. SPONTANEOUS HYPOPOTASSEMIA, HYPOMAGNESEMIA, ALKALOSIS, AND TETANY: METABOLIC AND PHYSIOLOGIC STUDIES

IVAN J. MADER, M.D. (BY INVITATION), AND LLOYD T. ISERI, M.D., DETROIT, MICH.

A 33-year-old woman presented herself with tetany, muscle pain, weakness, paresthesia, hypertension, and nervousness. There was no history of vomiting, diarrhea, or use of medicaments. The plasma showed K—1.60, Na—143, Mg—1.12, Ca—3.95, CO_2—43, Cl—84, P—1.6 meq./L., and pH, 7.5. Trousseau sign remained positive even after intravenous calcium gluconate and gradually became negative after prolonged KCl therapy. Detailed corticoid excretion studies revealed high absolute values for 17-OH-corticoid derivatives (45.9 mg. per gram of creatinine excreted) consistent with hyperfunctioning adrenal cortex. The glucose tolerance test showed delayed return of glucose level to normal. Sweat Na concentration was abnormally low but the paradoxical DOCA test was negative. Initial renal function studies revealed depressed GFR to 55.5 ml./min. but this gradually became normal over a four-month period in spite of persistent hypopotassemia. The initially low filtration fraction became elevated reflecting the underlying vascular hypertensive disease. The lowest U/P ratio for K attained was 5.2 with plasma K of 1.84 meq./L. The highest tubular K reabsorption attained was 1.12 meq./min. (46 per cent of amount filtered). Sodium load increased the K-U/P ratio, C_K, and C_K/C_{In} ratio. Potassium load increased the Ck and Ck/C_{In} ratio, but only slightly increased the K-U/P ratio. Complete metabolic studies demonstrated (1) failure of correction of hypopotassemia with 50 meq. K/day intake, (2) marked negative Na balance and egress of Na out of cells during

high K intake and balance (1.6 meq. K = 1.0 meq. Na), (3) a slightly negative magnesium balance on 12 meq. Mg/day intake despite low serum Mg level, (4) loss of K through both urine and stool, and (5) equilibrium of N balance. Infusion of saline caused a marked fall in serum Mg and plasma K without increasing urine Mg or K. Profound alteration of the EKG was only partially corrected by high intake of K.

These studies demonstrate a syndrome of spontaneous hypopotassemia and hypomagnesemia with alkalosis and tetany most likely due to adrenal cortical hyperfunction.

113. OUTPATIENT THERAPY WITH RESIN AND LOW-SODIUM DIET

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(Introduced by O. M. Helmer, Ph.D.)

This study was designed to determine if results comparable to those previously observed in hospitalized hypertensive patients treated with low sodium diets and resin could be obtained in outpatients and if any contraindications existed for prolonged therapy of this type. Sodium-removing resins were used in conjunction with dietary restriction of sodium, in order to accomplish greater sodium removal than could be produced by diet alone.

Twenty patients were treated with this regimen for periods of one year or longer. No significant untoward effects of prolonged resin therapy were noted.

Six patients, the subject of this report, were selected from the larger group on the basis of two criteria: (a) pretreatment observation periods of ten months or longer during which frequent outpatient blood pressures were obtained, and (b) average pretreatment *diastolic* blood pressures of 115 mm. Hg or higher (range 116 to 131).

Diet and resin dosage were adjusted to maintain 24-hour urine sodium (obtained at intervals of one to two weeks) below 5 meq. per 24 hours. Supplementary potassium dosage was adjusted to maintain adequate potassium balance as evaluated by urinary excretion of this cation.

During treatment periods of 12 to 35 months, blood pressures taken at intervals of one to two weeks indicated blood pressure changes as follows: −73/−28, −72/−31, −40/−22, −25/−6, −21/+1, −5/0. All patients improved symptomatically. Transverse cardiac diameter decreased 1 to 2.8 cm. Kidney function measurements remained essentially unchanged.

114. UNRECOGNIZED HYPOGLYCEMIA WITH SUBSEQUENT PARADOXICAL HYPERGLYCEMIA—AN IATROGENIC FACTOR CAUSING LABILITY IN DIABETES

Richard M. McKean, M.D., and Robert H. Hamburg, M.D. (By Invitation), Detroit, Mich.

This paper uses material obtained during a three months' hospitalization of a labile diabetic to demonstrate the phenomenon of an unrecognized hypoglycemia followed by a consequent and seemingly paradoxical hyperglycemia. The patient was a 47-year-old white man, diabetic for 17 years, who was brittlely "controlled" on 105 units of insulin per day. Signs of an unnoted convulsion and an x-ray demonstration of new compression fractures of the dorsal spine led to a diagnosis of an unrecognized hypoglycemia. Blood sugars taken every two hours round the clock revealed a recurring pattern

of severe hypoglycemia followed by acute rises to markedly hyperglycemic levels which persisted from 12 to 72 hours. These marked variations cleared only when the insulin dosage had been reduced to less than 50 units per day. Thus too much insulin had caused a significant and initially unrecognized hypoglycemia which in turn marshalled the homeostatic mechanisms with the subsequent production of a relatively insulin-resistant, paradoxical hyperglycemia. Said hyperglycemia might well lead the clinician to order further increases in insulin dosage. Causes of this pattern may be (1) an increased insulin dosage to control infection or other stress without a decreasing dosage when the stress clears; (2) too rapid increases in insulin dosage to get ahead of a frankly decompensated diabetes; or (3) attempts to span the whole 24 hours with a single insulin dosage. Adrenal medullary stimulation with the production of large amounts of epinephrine leading to mobilization of the liver glycogen stores and stimulation of the pituitary adrenal axis with cortical hormone elaboration with its known hyperglycemic action are two of the possible mechanisms involved. Recognition of this phenomenon requires the obtaining of frequent and especially nocturnal blood sugar determinations. When the diagnosis is made, treatment consists of a substantial reduction in insulin dosage with changes in time of administration and proportions of the long- and short-acting insulins plus appropriate changes in the dietary prescription.

115. PRELIMINARY TRIAL OF NEW ORAL NONMERCURIAL DIURETIC, SC-3858

W. P. McKeever, M.D., C. R. Hines, M.D., and I. C. Winter, M.D., Chicago, Ill.

(Introduced by A. R. Colwell, M.D.)

Experiments with dogs and rats have indicated SC-3858 (2-amino-4-hydroxy-5-methyl-6-phenyl pyrimidine) to be a potent oral diuretic. Based on Lipschütz's method, its potency in rats approaches that of parenteral Salyrgan.

Twenty-eight trials of SC-3858 and Mercuhydrin (for comparison) were conducted on five patients with varying degrees of congestive heart failure. These patients were carefully followed on a metabolic balance study.

The dosage of SC-3858 (given in tablet form) varied during the several studies. Initially 1.0 Gm. daily in divided doses for three days was tested. Later single doses of 0.5 and 1.0 Gm. were given in an effort to obtain dosage response curves. Mercuhydrin in 1.0 and 2.0 ml. amounts was given parenterally.

Diuresis was arbitrarily defined as a 24-hour urinary sodium excretion greater than three times the standard deviation of the mean excretion during the control periods. SC-3858 was diuretic in three of the five patients according to this criterion. Four of the five responded to Mercuhydrin. Sodium excretion exceeded chloride, giving a sodium:chloride ratio of 1.12; whereas the same ratio for Mercuhydrin was 0.75. This suggests a different mode of action from mercurial diuretics. In general, the response in terms of sodium excretion produced by 1.0 Gm. of SC-3858 was less than that following 1.0 ml. of parenteral Mercuhydrin.

Hourly urine collections following single doses revealed that the onset of sodium diuresis occurred 4 to 5 hours after oral administration and persisted for 5 to 6 hours. These findings correspond closely to animal (dog) results.

Two patients experienced mild gastrointestinal side effects. Serial studies of hepatic, renal, cardiovascular, and hematopoietic function revealed no evidence of toxicity.

It is concluded that SC-3858 is a diuretic in human beings with heart failure of varied etiology, probably acts in a different manner from Mercuhydrin, and at the dosage tested is less potent than 1 ml. of Mercuhydrin.

116. CIRCULATORY CHANGES CAUSED BY MULTIPLE PULMONARY A-V FISTULAS

WILLIAM F. MILLER, M.D. (BY INVITATION), AND CARLETON B. CHAPMAN, M.D., DALLAS, TEX.

Physiologic studies were done on a patient with multiple pulmonary A-V fistulas, proved later at autopsy, in order to elucidate the nature of the associated functional derangement. In addition to the classical features of the disorder, the patient had a weakly pulsating aneurysmal mass on the back of the left chest.

At rest, peripheral arterial O_2 saturation varied from 65 to 77 per cent, the patient breathing room air. On pure O_2, the corresponding value was usually 100 per cent. Exhaustive pulmonary function studies disclosed no evidence of significant primary pulmonary disease.

Mild exercise caused decrease in peripheral O_2 saturation, whether the inspired gas was room air or pure O_2.

Extensive studies on the aneurysmal mass disclosed that it was composed of widely dilated systemic arterial channels not directly connected with the pulmonary circuit.

Cardiac catheterization showed normal RV and PA pressures whether room air or pure O_2 was given. There was no evidence of intracardiac shunting of blood. The resting cardiac output (room air) was 9.2 L./min., and was slightly reduced during pure O_2 breathing, as judged by direct Fick and dye dilution measurements.

Polarographic measurement of arterial pO_2 disclosed a right-left shunt of 35 per cent and an A-a pO_2 difference of 464 mm. when pure O_2 was given.

The data suggest that pulmonary hemodynamics were little affected by pure oxygen breathing and that the apparent abolition of the shunt by it may have been a dissolved oxygen effect. The shunt was shown to be intrapulmonary, owing to the presence of a huge number of dilated pulmonary artery branches. The findings at autopsy differed from those in the literature in that only a few of the fistulous channels were visible to the naked eye. The functional and clinical implications of shunts of this type are to be discussed.

117. HIGH FAT DIET IN CASES OF FATTY LIVER (AND OTHER HEPATIC DISORDERS)

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(INTRODUCED BY LEON SCHIFF, M.D.)

A high fat diet has been used experimentally in the treatment of patients with liver disease admitted to Cincinnati General Hospital during the past two years (1953 and 1954). The diet has been the regular hospital diet to which fat supplements were added to provide daily 300 Gm. of fat, 100 to 120 Gm. of protein, and 250 to 300 Gm. of carbohydrate. Caloric intake was in excess of 4,000 calories per day. Intake of diet and supplements was observed. Random stool fat determinations were done to verify fat absorption.

Eight cases with fatty liver and eight cases of long-standing nutritional cirrhosis received the high fat diet. Nine cases of infectious hepatitis were

given the high fat regime while alternate cases were given a low fat diet. One case of liver coma was managed with the high fat diet.

In the cases with fatty livers there was progressive clinical improvement, regression of liver size, improvement of liver function, and in three cases where serial biopsies were done, visible fat was virtually gone in four weeks.

The cirrhotics improved clinically and also from the laboratory standpoint. In the cases of infectious hepatitis there was little difference between the treated and the control groups. Weight gain occurred in those patients treated with the high fat diet. One case of serum hepatitis not in this series was treated during the entire incubation period with the high fat diet. There was no change in the course of the patient in liver coma.

Minor temporary gastrointestinal disturbances were the only complications from the high fat diet. Blood lipid determinations showed no consistent alterations. Total lipids as well as phospholipids, neutral fat, and fatty acid generally remained within normal range. Cholesterol and cholesterol ester values varied considerably from patient to patient.

The results indicate that patients with acute or chronic liver disease can absorb and utilize large amounts of fat. The implications in regard to fatty liver will be discussed.

118. CHARACTERISTICS OF INFLUENZA A AND B VIRUSES ISOLATED AND PROPAGATED IN CULTURES OF HUMAN EMBRYONIC TISSUES

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(Introduced by Clayton G. Loosli, M.D.)

The influenza viruses have been studied quite extensively in various animal hosts and in the cells thereof in tissue culture; however, little information has been acquired regarding their behavior or characteristics when infecting human tissues. An approach to this problem is provided by the use of embryonic human lung and kidney cultures in which virus can be isolated directly from man, thereby excluding any possible effects of animal host passage.

On initial isolation from nasal washings, the presence of virus in tissue culture fluids could be detected by hemagglutination from 3 to 7 days after inoculation and could then be identified serologically by hemagglutination-inhibition. By successive passages the time of appearance of hemagglutinins was shortened to 24 hours. Strains of influenza A (1953) virus agglutinated human type O erythrocytes but failed to agglutinate fowl erythrocytes at 22° C. until at least 5 tissue culture passages had been made. Influenza B (1954) strains agglutinated both types of erythrocytes in equal titer on initial isolation. No increase in the ability of either virus to grow in the allantoic sac of the chick embryo occurred after human tissue culture passages. The antigenic structure of these viruses as compared to the same strains isolated and propagated in the chick embryo was similar, and antibody levels as measured by hemagglutination-inhibition with acute and convalescent human sera revealed little deviation between the results obtained with virus propagated in the chick embryo or in human tissue culture.

Differences between the growth of virus in explant cultures of lung or kidney tissue were not apparent. However, prior digestion of kidney tissue with trypsin resulted in cultures with a greater number of epithelial cells in which higher virus titers were attained after inoculation. By frequent changes of nutrient media in these cultures, successive periods of maximum virus titer

could be demonstrated. Regardless of culture technique and with both influenza A and B strains, epithelial cell destruction was associated with growth of virus. Only occasionally were all epithelial cells affected in a culture and virus production continued as long as there were intact cells present.

Thus, human embryonic lung or kidney in culture serves as a convenient system for isolation and propagation of influenza viruses, and the inference can be made that the characteristics and behavior of the viruses are similar in the infected human host.

119. THE SINGLE INTRANASAL APPLICATION OF VITAMIN B_{12} CRYSTALS IN PERNICIOUS ANEMIA

A Clinical and Laboratory Appraisal of Two Patients

Raymond W. Monto, M.D., John W. Rebuck, M.D. (By Invitation), and James T. Howell, M.D. (By Invitation), Detroit, Mich.

A single application of crystalline vitamin B_{12} to the nasal mucosae can be demonstrated to produce a clinical and laboratory response in pernicious anemia patients. A complete clinical response can be obtained, as we have shown, with 150 and 200 μg of vitamin B_{12} crystals. The effects of a single application of crystalline vitamin B_{12} to the nasal mucosa in two patients with pernicious anemia were studied.

The first patient with an initial erythrocyte count of 2.2 millions received under direct vision 150 μg of B_{12} crystals on the nasal mucosa and demonstrated a reticulocytosis of 44.5 per cent on the sixth day. A complete clinical and hematological remission (hemoglobin 16.5 Gm.; red blood count 5.65 millions) was obtained for a three-month period without further treatment. Assay of the urine for an eight-hour interval after treatment showed significant increase in B_{12} activity.

A further observation revealed that the instillation of nasal B_{12} washings from a normal subject into this patient's stomach prior to treatment failed to produce reticulocytosis in a twelve-day test period.

A second patient in relapse with an erythrocyte count of 2.01 millions was given 200 μg B_{12} crystals on the nasal mucous membrane. A reticulocytosis of 43.4 per cent on the seventh day was encountered. A complete clinical remission was obtained with an increase of erythrocytes to 4.12 millions in a two-month period without further treatment. Bio-assay of the urine showed an excretion of 12 per cent of the B_{12} administered.

It is concluded that a single application of vitamin B_{12} to the nasal mucosae has effected complete clinical and laboratory response in these two pernicious anemia patients. The B_{12} crystals are rapidly absorbed by the nasal mucous membranes and are nonirritating. We have also noted that the absorption of the vitamin B_{12} by the nasal mucosa is probably not dependent upon binding with "intrinsic factor."

120. SPECIFIC ALLERGIC MANAGEMENT OF ACUTE RHEUMATIC FEVER

William M. Mount, M.D., Crawfordsville, Ind.

(Introduced by Theron G. Randolph, M.D.)

The allergic nature of the tissue response in rheumatic fever, recognized by both cardiologists and pathologists, has usually been attributed to sensitization to the streptococcus. From observations to be reported herewith, it ap-

pears probable that other etiologic agents in addition to the streptococcus are involved and that rheumatic fever is basically an allergic disease similar in many respects to other clinically recognized allergic manifestations. In my experience, management from this standpoint with a careful consideration of the roles of multiple causative allergens is associated with a more prompt control of the clinical manifestations of the disease than may be accomplished from alternative modes of therapy.

The frequent occurrence of the rheumatic fever syndrome in allergy patients and their families, and vice versa, as well as common physical findings in both groups have been noted. Also the unusually high reported incidence of aspirin sensitivity as well as certain other patterns of reaction in rheumatic fever cannot be accounted for other than by the behavior of an allergic manifestation.

In the allergic management of rheumatic fever, a detailed history is taken and skin tests are performed with inhalant allergens. Daily treatment with small doses of the clinically significant skin test positive allergens—most commonly house dust and fungi—is continued during the acute phase. Diet control for possible food sensitivity is instituted. Because of the frequent importance of bacterial sensitization, foci are eradicated and vaccine therapy is often necessary for complete recovery.

In four typical and fifteen atypical primary and recurrent rheumatic syndromes normal temperature, absence of joint manifestations and a marked general clinical improvement has been attained in as little as seventy-two hours. The sedimentation rate has fallen rapidly, reaching normal in less than three weeks. In eight days the P-R interval has changed from 0.24 to 0.16 second.

This approach to rheumatic fever also offers much in the prophylaxis of initial and recurrent attacks in contrast to the inherent dangers in the prophylactic use of sulfonamides and penicillin. These drugs not only frequently sensitize but may be responsible for inducing allergic syndromes, including recurrences of rheumatic fever.

121. SERIAL KIDNEY BIOPSY STUDIES IN PATIENTS WITH THE NEPHROTIC SYNDROME

ROBERT C. MUEHRCKE, M.D. (BY INVITATION), ROBERT M. KARK, F.R.C.P., CONRAD L. PIRANI, M.D. (BY INVITATION), AND JAMES A. SCHOENBERGER, M.D. (BY INVITATION), CHICAGO, ILL.

Fourteen patients, aged 13 to 66 years, who had the nephrotic syndrome, were studied clinically by means of needle kidney biopsy and by discrete and gross renal function tests. Ten patients were studied during the edematous phase while four patients were studied after the edema subsided. Serial kidney biopsies have been obtained to date in eight patients.

The histologic diagnoses were: subacute glomerulonephritis, seven cases; chronic glomerulonephritis, one case; amyloidosis, one case; and tubular disease of undetermined type, two cases. Essentially normal biopsies were found in three patients who were studied while in clinical remission. One of two patients with undetermined tubular disease died in uremia three months after biopsy; an autopsy disclosed bilateral renal vein thrombosis.

Serial biopsy studies in patients with nephrosis, due to subacute glomerulonephritis, revealed the earliest morphologic lesion to be a moderate to severe fatty degeneration of the tubular cells with essentially normal glomeruli. Subsequent biopsies showed either thickening of the capillary glomerular

basement membrane or increased cellularity of the glomeruli with epithelial crescents. When peripheral edema subsided and azotemia developed, as a rule biopsy studies disclosed early glomerular hyalinization and marked interstitial fibrosis with marked tubular atrophy.

There was no correlation between the degree of proteinuria and extent of glomerular involvement. In some cases, the degree of proteinuria was inversely related to the degree of glomerular damage. In this respect, in a series of 130 biopsies, proteinaceous material was seen in Bowman's space in 33 instances, but in 11 of these patients there was no proteinuria. This observation confirms the concept that protein is filtered by the glomeruli and reabsorbed by the tubules.

Discrete renal functions were reduced in all edematous patients. In most cases there was an excellent correlation between renal function tests and structural changes. However, the "effective renal plasma flow" was often diminished out of proportion to the histologic alteration of the kidney. All these findings will be discussed with respect to the pathophysiology of the nephrotic syndrome.

122. AGGLUTINATION OF ERYTHROCYTES IN VITRO INDUCED BY HYDRAZINE, INCLUDING A POSITIVE COOMBS TEST

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Previous work in this laboratory has demonstrated the development of positive direct and indirect Coombs tests in the dog following intravenous phenylhydrazine. The direct Coombs test became positive in 2 days and remained positive for 2 to 4 weeks. The phenomenon was considered nonimmune because of its sudden onset, short-lived status, and the lack of potentiation by subsequent doses of the drug. Attempts to demonstrate the positive direct Coombs test by action of phenylhydrazine in the test tube were inconclusive. By substituting hydrazine in the test tube it has been possible to demonstrate agglutinative and hemolytic phenomena similar to those observed with phenylhydrazine in vivo.

Canine, human, and rabbit erythrocytes washed 6 times with saline were used. A 2 per cent solution of hydrazine sulfate in normal saline was serially diluted with saline. One-tenth milliliter of the hydrazine concentrations was mixed with 0.1 ml. of a 2 per cent suspension of washed R.B.C. and allowed to stand at room temperature for 30 minutes. Complete hemolysis occurred in dilutions through 1:16. Hemolysis and agglutination occurred between dilutions 1:8 and 1:32. Agglutination without hemolysis occurred between dilutions 1:32 to 1:128. Beyond these dilutions the gross inspection yielded no agglutination. The cells were washed 3 times in abundant saline, and upon the performance of the direct Coombs test positive results were detected with 8 out of 16 erythrocytes obtained from 7 dogs, 8 human beings, and 1 rabbit.

Hydrazine in increasing dilutions interacts with washed erythrocytes from different species in the test tube and causes the sequence of hemolysis, hemolysis and agglutination, agglutination, and a positive Coombs test.

Three possibilities for the mode of action of hydrazine in evoking a positive direct Coombs test have been considered: (1) exposure of proteins in the erythrocytes which are capable of interaction with the canine serum antibodies in the Coombs serum; (2) alteration of the surface of the erythrocytes so as to cause the adsorption of normal proteins; and (3) the chemical acting as a coupling agent between the surface of erythrocytes and normal proteins.

123. EFFECT OF A RIBOFLAVIN ANTAGONIST UPON LEUKOCYTES OF NORMAL AND SHAY MYELOID CHLOROLEUKEMIC RATS

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An analog of riboflavin, 6,7-dimethyl-9-(2′acetoxy-ethyl) isoalloxazine (U-2112), has been shown to be a reversible antagonist of riboflavin when administered orally or parenterally to normal rats and to inhibit the Murphy-Sturm lymphosarcoma and Walker adenocarcinoma 256 in rats.

When given to normal rats ingesting a riboflavin-containing complete diet, a dose of U-2112 insufficient to interfere with growth or weight gain resulted in significant granulocytosis without lymphopenia. Total leukocyte counts rose from an average of 20,000 per cubic millimeter to 32,000 and granulocytes from 3,300 to about 11,000. There was no significant difference between males and females. The changes were highly significant when compared with blank-treated controls.

When U-2112 was administered similarly to rats bearing the Shay myeloid chloroleukemia, there was no beneficial effect on the course of the leukemia, but rather a tendency (not statistically significant) for the treated animals to have larger numbers of abnormal granulocytes than blank-injected control animals. In contrast, TEPA induced rapid and prolonged remissions in the leukemic rats.

The correlation between the effects of the analog on normal and leukemic granulocytes and the different responses of different malignant conditions indicate an ability of the analog to interfere specifically with vital metabolic processes of certain malignant cells.

124. OCCURRENCE OF ABNORMAL HEMOGLOBIN E IN THAILAND

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Hemoglobin E, a newly recognized abnormal form of hemoglobin, was detected by the authors when they were studying the genetic background of a Thai patient with Mediterranean anemia, only one of whose parents had the Mediterranean trait. Hemoglobin E was then identified in other Thai persons, but could not be found in the blood of several hundred subjects of Caucasian, Negro, or American Indian ancestry. The present study was undertaken to determine the incidence of hemoglobin E among the Thais.

Blood specimens were collected from 590 individuals in Thailand; hemoglobin E was found in 74 instances (12.5 per cent). Analyses were made by paper electrophoresis between ½ inch glass plates, employing a 0.06 molar veronal buffer of pH 8.8. The controlled electrical output of the power supply was 350 volts and 12 to 15 milliamperes. Hemoglobin E has a mobility intermediate between hemoglobins C and S. Subjects were selected at random except that an attempt was made to sample both the "normal" and the patient population. Results are tabulated in the accompanying table.

	NO. STUDIED	NO. WITH HbE	PER CENT WITH HbE
A. Males			
Medical students and staff	171	14	8.2
Patients	166	17	10.2
B. Females			
Nurses, medical students and staff	170	26	15.3
Patients	83	17	20.5

Any conclusion about the apparently greater incidence of hemoglobin E in women is being withheld until more cases have been studied.

All but two of these 74 subjects were classified as having hemoglobin E trait; the concentration of hemoglobin A (adult normal hemoglobin) was greater in their bloods than hemoglobin E. The remaining two patients were found to have only hemoglobin E plus a small amount (2.6 and 6.0 per cent) of fetal hemoglobin, and were classified as having hemoglobin E disease. One of these patients was a man, 25 years of age, who was seen for the chief complaint of bronchitis; the second was a man, 37 years of age, who was being treated for a peptic ulcer.

The subjects in whom hemoglobin E was found came from different areas of Thailand. As far as could be determined all had racial backgrounds of Thai ancestry although some had an admixture with Chinese parentage.

125. THE USE OF BEER IN THE LOW SALT DIET WITH SPECIAL REFERENCE TO RENAL DISEASE

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Because of the high caloric, low protein, and low sodium content of beer, it was our purpose to determine if beer could be used in the low salt diet in renal disease without unfavorable effect on the cardiovascular-renal system.

The study occupied a period of over one year. Five patients with chronic glomerulonephritis, two with Kimmelstiel-Wilson's disease, and two with malignant hypertension were studied. These patients were placed on a regular hospital 250 or 500 milligram sodium diet for a period of three days. Following this, they were placed on similar diets with one-fourth to one-third of calories obtained as beer (45 mg. sodium) for a period of five or ten days. Fluid intake was limited to 2,000 ml. daily during both periods. Studies of serum nonprotein nitrogen, bicarbonate, chlorides, sodium, and potassium were measured on alternate days throughout both periods. Similarly, daily urinary outputs were measured for sodium, potassium, chloride, and 24-hour albumin determinations. An Addis count was done on six patients during the first period and at the end of the period with the beer-included diet. Phenolsulfonphthalein excretion studies and renal clearance studies were done on six cases during the first period and at the conclusion of the beer-included diet. Urinary output, weight, and blood pressure were measured daily throughout both periods. One patient with chronic glomerulonephritis and the nephrotic syndrome was placed on a 500 milligram sodium diet which included 1,536 ml. beer (2.5 mg. sodium per 100 ml.) for a continuous period of one year. Similar studies were performed on this patient at the beginning of the beer-included diet and after the patient had been on the beer-included diet one year.

It was concluded that in patients with chronic nephritis with or without the nephrotic syndrome, in malignant hypertension, and in other renal diseases studied, there was no evidence either from a clinical or laboratory standpoint that inclusion of beer in the low salt diet caused any unfavorable response. The diet itself appears to be more palatable for some patients than the regular low salt diet frequently used in renal diseases.

126. THE USE OF ADDITIONAL CHEST LEADS TO ESTIMATE THE SIGNIFICANCE OF ELECTROCARDIOGRAMS THAT SUGGEST INCOMPLETE RIGHT BUNDLE BRANCH BLOCK

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The frequent occurrence of late secondary R waves in precordial leads taken over the right precordium, without significant prolongation of the QRS interval, has raised legitimate doubts regarding the significance of incomplete right bundle branch block in the electrocardiogram. When the history and cardiac findings are known to be normal, these electrocardiographic changes may be safely ignored, but if clinical data are inadequate, interpretation of the findings in question may be very difficult. For this reason, additional chest leads have been taken on a number of individuals, some with normal hearts and several with known cardiac abnormalities, in an attempt to determine whether the location of the late secondary R waves may be of value in determining the significance of this finding. The additional leads have been taken in two planes: (1) to the right of the usual precordial leads, i.e., V_3, V_4, and V_5 on the right; and (2) above the usual right precordial leads, i.e., V_1 and V_2 in the second and third intercostal spaces.

In general, it can be concluded that in normal individuals, late secondary R waves are commonly found in leads above the usual V_1 and V_2 positions but are absent in leads taken to the right of the usual precordial sites. This finding suggests that the late R waves here may result from physiologic late activation of the base of the right ventricle and not from right branch block. In most individuals with abnormal hearts, late secondary R waves are found in leads to the right of those customarily taken but may be found in leads taken higher up as well. Although the significance of tracings that suggest incomplete right bundle branch block must often be evaluated by the clinical findings, the additional chest leads described may prove to be of considerable value.

127. THE EFFECTS OF HISTAMINE AND REGITINE ON THE PULMONARY ARTERY PRESSURE IN A CASE OF PHEOCHROMOCYTOMA

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WITH THE TECHNICAL ASSISTANCE OF A. BENNETT, B.A. (BY INVITATION), AND M. FINLEY, B.S. (BY INVITATION)

Cardiac catheterization was done on a patient whose clinical studies justified the diagnosis of pheochromocytoma. The effects of both histamine and regitine on the pulmonary artery pressure were determined. These studies were done before, and 2 and 10 weeks following, the removal of a pheochromocytoma. At no time was there evidence of congestive heart failure.

Before removal of the pheochromocytoma, the superior venal caval, right atrial, and right ventricular pressures were normal. The pulmonary artery, pulmonary "capillary." and systemic arterial pressures were slightly elevated. Injection of histamine produced a marked rise in systemic, pulmonary artery,

and pulmonary "capillary" pressures, and the injection of regitine immediately reversed this pressor effect on these three segments of the vascular bed.

Two weeks following removal of the pheochromocytoma pulmonary artery and pulmonary "capillary" pressures were normal and the systemic arterial pressure was slightly elevated although it had been within normal limits most of the time during the two weeks following surgery. Although there was a very slight rise in pulmonary artery pressure following histamine injection, it is considered probably of insignificant amount. Injection of regitine was followed by a fall of pressures to the previous postoperative levels.

Ten weeks after the operation systemic arterial pressures were persistently elevated but the pulmonary artery pressures initially were within normal limits. Following the injection of 0.025 mg. histamine base there was no significant elevation of either the pulmonary artery or systemic arterial pressures.

The injection of 0.05 mg. histamine base produced a probably insignificant rise in both systemic arterial and pulmonary artery pressures.

These data demonstrate that the endogenously produced pressor substance in a patient with pheochromocytoma is associated with a marked elevation in pulmonary artery pressure. It is postulated that the measurements of pulmonary artery pressure during a period of persistently high output of pressor substance as well as during a spontaneous paroxysm of hypertension and during a histamine-induced paroxysm of hypertension may be of value in the differentiation of hypertension due to pheochromocytoma from that of essential hypertension.

128. THE DIURNAL VARIATION OF THE SERUM IRON LEVEL

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(Introduced by G. E. Burch)

Vahlquist in 1941 first described the diurnal variation of the serum iron level—falling by day and rising by night. It was soon recognized that the nocturnal rise was related to sleep and that the rhythm becomes reversed in the night worker who sleeps by day. The present writer has been interested in the mechanism of the variation and has observed its behavior in normal individuals under various conditions and in a variety of diseases. The principal conclusions are that the diurnal variation is independent of the eating of meals, physical exercise, and the body temperature. Nor is it due to changes in blood pH. Hormonal stimulation (adrenalin, ACTH, and cortisone) in the normal individual is apparently without effect and a diurnal variation occurs in Addison's disease and Simmonds' disease, as it does also in thyrotoxicosis and myxedema. (This does not exclude the possibility of a change in the rate of iron turnover as a result of such stimulation.) In erythropoietic disorders a failure of the iron level to rise during sleep is a regular accompaniment of increased erythropoietic activity. In normal individuals the rate of removal of radioactive iron from the plasma is greater by day than by night. No such difference was noted in a patient with iron-deficiency anemia. These findings suggest that the diurnal variation of the serum iron level is dependent upon different levels of erythropoietic activity by day and by night and imply that hemoglobin synthesis is slowed down during sleep.

129. URINARY TRACE METALS FROM NORMAL AND TREATED AND UNTREATED HYPERTENSIVE PATIENTS

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Because hydrazinophthalazine, azide, thiocyanate, ethylenediamine tetraacetate, and mercaptans are vasodepressor in human hypertension and in renal hypertensive rats, and because these diverse substances have a common affinity for transition elements, 24-hour urine from 6 normal controls and 13 untreated and treated (by hydrazinophthalazine) hypertensive patients were analyzed spectrographically. Twenty-two elements were sought using cathode-layer, direct-current excitation. Sodium, magnesium, aluminum, silicon, phosphorus, potassium, calcium, iron, copper, and strontium were demonstrated in all 31 specimens as well as in caprylic alcohol and nitric acid control solutions used in the method. Of the remaining 12, chromium appeared 20, titanium 13, silver 12, manganese 5, cobalt 4, and cadmium 4 times; vanadium, lead, and nickel occurred thrice, and zinc, arsenic, and molybdenum once.

In the urine of all of 5 cases of severe untreated hypertension, elements with atomic numbers 22-30, excluding omnipresent copper and iron, were found (10 times); after 10 days of hydrazinophthalazine they were present in 3 cases (6 times). Of 5 other patients treated for a year, 2 excreted these elements (8 times). Of 5 normal urines, 2 contained them (3 times). Moreover, cadmium, excreted by one of 5 controls, disappeared from 3 hypertensive urines after treatment. Specific differences between normalcy and untreated hypertension were difficult to demonstrate; however, chromium, for which the method was quite sensitive, appeared in all of 5 hypertensive and only 2 of 5 normal specimens, and titanium and cadmium were more frequent in hypertensive urines. Not only were there fewer elements excreted in normotension but this essentially qualitative analysis suggested less of those than were present.

These results indicate that certain trace metals occur in urine, that severe untreated hypertensive patients excrete more than normal amounts of chromium and perhaps cadmium and titanium, and that treatment with substituted hydrazine appears to lessen excretion of several metals with atomic numbers 22-30.

130. IN VITRO STUDIES ON METHEMOGLOBIN REDUCTION AND GLYCOLYSIS BY ERYTHROCYTES IN CONGENITAL AND ACQUIRED METHEMOGLOBINEMIA

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(Introduced by William W. Engstrom, M.D.)

It has been pointed out previously (Eder, Finch, and McKee, J. Clin. Invest. 28: 265, 1949) that congenital methemoglobinemia is an inherited biochemical defect attributable to a lack of a factor which in normal erythrocytes reduces constantly produced methemoglobin to hemoglobin. It is also known (Drabkin, Federation Proc. 5: 132, 1946) that glucose is required for the normal conversion of methemoglobin to hemoglobin. In this study, the ability of erythrocytes to effect glycolysis and reduction of methemoglobin was examined in patients with congenital methemoglobinemia, in acquired methemoglobinemia, and in normal subjects.

Erythrocytes were obtained from three patients from the same family with congenital methemoglobinemia and from one patient with acquired methemoglobinemia due to acetanilid intoxication. In other in vitro studies methemoglobin was artificially induced in normal erythrocytes with a solution of sodium nitrite.

Erythrocytes containing methemoglobin were incubated at 37° C. with solutions of glutathione hydrochloride, cysteine hydrochloride, ascorbic acid, normal plasma, and glucose. Samples were removed at one- to two-hour intervals and tested for methemoglobin by the method of Evelyn and Malloy. In all erythrocytes, glutathione, cysteine, and ascorbic acid (all reducing substances) had only a slight transient action on the conversion of methemoglobin to hemoglobin, which reverted back to methemoglobin within two hours. In acquired or induced methemoglobinemia complete reduction of methemoglobin occurred only in the presence of glucose or plasma. However, the methemoglobin in the erythrocytes from patients with congenital methemoglobinemia remained elevated with 24 hours of incubation with glucose or plasma. These erythrocytes, treated with sodium nitrite and resuspended in their own plasma or in glucose, displayed a hemoglobin-conversion mechanism in that the prenitritization concentration of methemoglobin was restored in 24 hours.

The rate of glycolysis was studied when saline-washed erythrocytes from normal subjects and patients with congenital methemoglobinemia were suspended in standard solutions of glucose in saline. All erythrocytes consumed glucose and produced lactic acid at comparable rates in vitro. Thus, it has been demonstrated that in congenital methemoglobinemia, the congenital deficiency in the methemoglobin reduction mechanism was not accompanied by a defect in utilization of glucose.

131. STEVENS-JOHNSON SYNDROME: CLINICAL STUDY OF NINE CASES INCLUDING TWO TREATED WITH ACTH AND CORTISONE

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Certain diseases of unknown etiology characterized by involvement of skin, mucous membranes, and joints (Reiter's syndrome, Stevens-Johnson syndrome, Behçet's syndrome, etc.) recently have been grouped together as the so-called oculomucocutaneous syndrome. We are of the opinion that these disorders are separate entities.

In the past eight years nine cases have been observed which fulfilled strict diagnostic criteria for Stevens-Johnson syndrome. These patients had two significant findings in common: a toxic, febrile onset of symptoms, and painful oral lesions which interfered with eating. No etiological agent was demonstrated in any of these patients. None presented evidence of joint involvement, antecedent diarrhea, or severe progressive eye involvement. This condition is characterized by recurrences, but, in general, the episodes are self-limiting.

In the past year two patients were treated with hormones. One received oral cortisone, and a second patient received both ACTH (Gel-form) and oral cortisone. These drugs had a prompt and favorable action on the toxic, febrile, and inflammatory aspects of the disease; the patients were able to take food, and felt much better. However, these substances did not significantly alter the basic course of this self-limiting condition.

132. THE RELATION BETWEEN DEEPLY INVERTED T WAVES IN THE MIDPRECORDIAL ELECTROCARDIOGRAM AND SUBENDOCARDIAL MYOCARDIAL INFARCTION

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On the basis of theory and of evidence derived from experimental and clinical studies, acute subendocardial injury has been related justifiably to the electrocardiographic finding of depression of the RS-T segment in direct and semidirect leads from regions overlying the surface of the damaged ventricle. That the presence of such an injury, particularly in its subacute phase, also may find expression in deeply inverted T deflections in midprecordial leads is suggested by data reported herein.

A group of 62 patients was selected on the basis of the electrocardiographic finding of a T deflection in precordial lead V_3 inverted to a depth of at least 5 mm., and as deeply inverted as, or more deeply inverted than, TV_5.

Review of clinical data available concerning these patients disclosed a high incidence of coronary disease (69.4 per cent) and an unusual prevalence of the clinical picture of severe coronary insufficiency (45.1 per cent), as contrasted with more typical expressions of acute myocardial infarction (14.5 per cent) or of angina pectoris induced only by exertion (8.2 per cent).

Among these 62 patients were 21 whose electrocardiograms reverted over a period ranging from weeks to months to an essentially normal configuration. Twenty of these 21 patients had substantial clinical evidence, apart from the electrocardiograms, of myocardial infarction or of severe coronary insufficiency. Reversibility of T-wave inversion bears, therefore, a high degree of correlation with supporting clinical evidence of myocardial injury.

Among 12 cases in which electrocardiographic findings of the type under consideration were present, combined in 6 instances with minor QRS changes, there were 9 in which necropsy disclosed patches of subendocardial necrosis or scarring in the anterior or lateral walls of the left ventricle.

These lines of evidence converge to form a pattern: clinically, of severe coronary insufficiency; electrocardiographically, of deeply inverted T deflections in the midprecordial electrocardiogram; and pathologically, of subendocardial infarction. Projected into the realm of electrocardiographic theory, this pattern can be related to a subendocardial boundary of injured fibers overlain subsequently by a transmural zone of ischemic myocardium.

133. THE EFFECT OF BILE DEPRIVATION IN THE GROWING PUPPY

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An animal can be completely deprived of bile by a cholecystnephrostomy with ligation of the common duct since all of the bile is thereby diverted through the kidney and none enters the intestines. To study the effect of such a deprivation on growth and development, the operation was performed on puppies when they were 3 months old. These animals kept on a normal, but low fat diet, and given only vitamins A, D, and K parenterally, grew and matured normally. One of the dogs lived 9 months and another 13 months after the operation. The most outstanding effect of the operation was the rapid and severe hypoprothrombinemia. While in the adult dog the fall in prothrombin is gradual and may require one or two months after the operation

to reach the hemorrhagic level, in the 3-month-old puppy, a severe hypoprothrombinemia developed in less than ten days. Whereas the maintenance dose of vitamin K_1 in the adult dog is 0.5 μg per kilogram of body weight, in the puppy at the age of 3 months, it was about 10 μg. Three months after the operation, the maintenance dose dropped to 2.5 μg and, in six months, it decreased to 1.0 μg. On administering large amounts of bile (7.5 ml. per kilogram of body weight) the prothrombin concentration was greatly increased but not completely restored to normal. The vitamin A level of the blood also markedly decreased, but was promptly elevated by parenterally administered vitamin A. Although no vitamin E was given, the animals manifested no abnormality of the muscular system. The bone development likewise was normal. The absence of bile did not cause any noticeable digestive disturbance. In summary, an animal deprived of bile can grow and mature, provided vitamins A, K, and possibly D are supplied by injection.

134. SENSORY ASPECTS OF CEREBRAL ALLERGY

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The progressive stages of altered sense organ function occurring in allergic reactions, namely, hyperesthesia, paresthesia, and anesthesia, are closely related to the subdivisions of cerebral allergy differentiated on the basis of abnormalities of interoception and exteroception. Development of cerebral allergy is shown by experimental exposure of specifically sensitized individuals to allergenic foods and drugs or particular chemicals apparently acting similarly. However, compartments are by no means mutually exclusive, there being a considerable overlap between proprioceptive and exteroceptive effects as well as between stages in both acute and chronic reactions at any given time and an alternation between these divisions at different times.

Awareness of the progressive stages of cerebral allergy is facilitated by observing numerous responses per patient, for early effects are more evident in mild reactions and advanced effects in severe ones.

In chronic low-grade cerebral allergy, auditory images appear to be more effectively associated than visual images.

Although anosomia and ageusia are commonly recognized as allergic manifestations, transient numbness, analgesia, blindness, deafness, or combinations thereof have not only received insufficient emphasis as allergic phenomena but are usually interpreted a priorily as due to "hysteria." In bouts of advanced cerebral allergy, auditory perception is usually the last sensory function to be lost; it often remains as the only exteroceptive contact, but if the response is sufficiently intense all sensory function may be temporarily absent—the subject not only remaining unresponsive during the attack but also totally amnesic for events transpiring in it.

135. ALLERGIC TYPE REACTIONS TO INDUSTRIAL SOLVENTS AND LIQUID FUELS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

The observation that many patients living in or near the petroleum refinery and storage area of extreme northwestern Indiana remained chronically ill with specifically undiagnosed allergic type manifestations and that several others developed such acute reactions in traveling through this region that they chose to go 100 miles around it, prompted an investigation of the clinical effects of various petro-, chloro- and/or sulfo- chemicals in allergy patients.

Although long regarded as irritants, there is rapidly increasing evidence that these fumes and related materials to be described are particularly irritating to allergic individuals. It appears that they either incite localized or constitutional hypersensitivity-type reactions in their own right or act to augment some basic mechanism responsible for these manifestations. Whatever the modus operandi, there remains little doubt that these man-made materials are impinging on man's health.

There being no easy way to detect the existence of this subtile syndrome, and as different subjects exhibit widely varied but related facets of it, clinical experience as aided by a detailed questionnaire has been most helpful.

Inhalation of the fumes of fresh paint, gasoline or kerosene, refinery areas, fresh newsprint, and rubbing alcohol were found to be precipitating causes of allergic type manifestations, respectively, in 74, 61, 60, 51, and 25 of 80 selected individuals having high degrees of clinical intolerance to a wide range of petroleum and coal derivatives and related substances. Intolerance to evaporating paint may be related either to natural or organic solvents, as turpentine, pine oil, cedar fumes, and evergreen Christmas decorations are a few of the several pine products producing clinically similar effects, presumably on the basis that petroleum and coal deposits are said to be derived from primeval conifer forests.

In addition to these common materials, there are important selected exposures to evaporating solvents from adhesives, plastics, and synthetic rubber. Finally, persons of various occupations, such as beauticians, barbers, mechanics, machine operators and oil workers, electroplaters, dry cleaners, painters, printers, launderers, housewives, chemists, pharmacists, and physicians—to name only a few—are associated with the handling of solvents and fuels involving contactant, inhalant, ingestant, or injectant routes of exposure.

136. ALLERGIC TYPE REACTIONS TO MOSQUITO ABATEMENT FOGS AND MISTS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Fogging for the control of adult mosquitos is rapidly becoming an omnipresent man-made hazard for persons clinically intolerant to various petro-, chloro- and/or sulfo- chemicals. Although many chemical exposures may be dodged, a victim is helpless to avoid the unannounced nocturnal fogging of his bedroom.

Reactions may occur at all ages, including childhood, and are of various degrees of severity depending upon susceptibilty and extent of exposure. Low-grade responses consist of burning and smarting of eyes and lips, burning and erythema of skin and sometimes a pruritic dermatitis limited to exposed parts, rhinitis, a sense of suffocation, coughing, and bronchospasm. In more advanced acute reactions, respiratory symptoms tend to subside coincident with the development of fatigue, headache and myalgia. These manifestations may be followed in turn by nervousness, apprehension, hyperactivity, and muscular incoordination which sometimes merge into and are superseded by a stuporous depression characterized by crying, mental confusion, and lethargy. Significantly, minor reactions may be the precursors of more severe ones provided causative exposures are maintained.

Of six patients who have developed advanced cerebral reactions following the inhalation of DDT-fuel oil insecticide mists in their homes, three have been observed in states of altered consciousness associated with violent muscular contractions simulating epileptiform seizures. In general, such severe re-

actions are apt to be precipitated with little warning, for the preliminary sequential stages tend to be telescoped when a highly intolerant person receives a massive exposure. One anosmic person reported no warning. Several have been loath to tell their experiences for fear that they might be judged mentally disturbed.

One patient, known to be intolerant to sprayed fruits or vegetables, detected the characteristic fogging odor upon awakening immediately prior to an acute reaction. When seen a few minutes later, she was having acute asthma and complained of generalized muscle aching and headache. Although her region had not been fogged, an area one-quarter mile to the windward was being treated at the time. Similar reactions in this patient have been observed while driving through an area fogged twenty-four hours previously.

The responsible ingredient of insecticide fogs has not been identified.

137. ALLERGIC TYPE REACTIONS TO MOTOR EXHAUSTS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Inhalation of the exhausts of Diesel-burning motors, suspected by 80 per cent of eighty patients clinically intolerant to petro-, chloro- and/or sulfochemicals constitutes the most troublesome and hazardous outdoor exposure, but in terms of sheer volume, the exhausts of non-Diesel liquid fuel-burning motors contribute more significantly to metropolitan air pollution.

Relatively heavy automotive exhausts rarely extend above the twentieth floor as a cause of symptoms in Chicago, being most concentrated in areas at street and below street levels, on humid, rainy, or nonwindy days or in the presence of atmospheric inversion. Barring specific exhaust blasts, exposures are generally greatest when multilaned traffic grinds to a stop-light halt, for decelerating exhausts not only contain relatively more noxious uncombusted hydrocarbons, but compactness of tandem and adjacent vehicles limits dispersion. Many patients refuse to ride in conventional rear-engine Diesel buses or the back seats of passenger cars unless windows are open—but this actually facilitates ingress of exhaust blasts.

Susceptible persons may develop rhinitis, coughing, headache, nausea and abdominal cramps (''car sickness''), or cerebral reactions. Although headache is the most common, brain dysfunction is more dangerous; minor reactions often resulting in accident proneness, and greater ones to ''going to sleep at the wheel'' and ''drunkenness'' sans alcohol. Several patients subject to cataleptic bouts of somnolentia (sleep-drunkenness) when trapped in traffic directly behind a Diesel or fume-spouting gasoline vehicle, commonly infract traffic regulations in escaping because of the greater danger otherwise.

Similar reactions may account for certain dopey, indecisive, uncoordinated pedestrians wallowing in a gaseous haze of motor exhausts, coal smoke, and other metropolitan industrial pollutants. One bedridden Chicago mother of six farmed-out children living leeward to a truck route, who regained her health on moving to an electrically equipped country home, becomes so confused on returning that she refers constantly to her previously written itinerary to avoid becoming disoriented.

Avoidance of Diesel routes, driving windward in multilaned traffic, and stopping with a car's gap between vehicles is helpful, but venting of Diesel exhausts upward, employing more refined fuels, and designing automotive cowls to prevent exhaust scooping are needed.

138. ALLERGIC TYPE REACTIONS TO INDOOR UTILITY GAS AND OIL FUMES

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Escaping utility gas, fuel oil fumes, and the combustion products of gas, fuel oil, or coal constitute the major indoor inhalant exposures for individuals clinically intolerant to a wide range of petro-, chloro- and/or sulfo- chemicals. Utility gas or its unvented combustion products arising from the gas kitchen range and gas refrigerator, though new or in good mechanical order, presents the greatest problem, but other gas utilities are equally troublesome if installed on the same floor level.

Fifty patients, having diverse allergic type symptomatology consisting of respiratory symptoms, chronic fatigue, myalgia, arthralgia, headache, generalized edema, and cerebral manifestations varying from slowness of thought to stupor or mania, have experienced a significant degree of relief following the removal of their gas stoves, refrigerators, water heaters, clothes driers, and/or fuel oil heaters and other specifically incriminated petroleum derivatives. A previously installed gas furnace may sometimes be retained if properly vented and isolated. The degree of symptomatic improvement in these selected cases has more than justified the incurred expense, in the opinions of the subjects, and the relationship of gas exposure to symptoms has been repeatedly demonstrated when subsequently visiting gas-equipped homes.

Clinical intolerance to indoor gas and fuel oil exposures develops insidiously and at any age, characteristically producing cumulative effects. These facts plus the ubiquity of gas exposures in such homes makes this problem difficult to recognize and frequently leads to tragic misinterpretations. Clues leading one to suspect its existence are the presence of otherwise unexplained kitchen, utility room, basement, or nocturnal symptoms, a greater ability to detect the presence of escaping gas than one's associates and the presence of numerous positive answers to a questionnaire designed for the detection of the petroleum problem in general. Whether one is actually intolerant to gas-burning home utilities is best determined by establishing a residence for one or two weeks in an electrically equipped apartment or home and then returning to the former surroundings and observing the effects from both moves, having controlled other known causes of the symptomatology in question in both places. The futility of moving otherwise is obvious.

139. ALLERGIC TYPE REACTIONS TO CHEMICAL ADDITIVES OF FOODS AND DRUGS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Although it is well recognized that only pure test materials should be employed in establishing specific allergic diagnoses, it is not generally appreciated that foods and biological medicinals may be sufficiently contaminated chemically as a result of technological processes as to cause reactions in allergic individuals clinically intolerant to certain petro-, chloro- and/or sulfo-chemicals or their derivatives. Either the chemical contaminants or the uncontaminated natural materials may produce identical local and constitutional effects—namely, pruritus, rhinitis and asthma or diaphoresis, fatigue, myalgia, headache, and other cerebral manifestations.

As intolerance to both the contaminants and the substrate commonly coexist in the same patient, these must be differentiated by employing test materials (for ingestion or injection) having a known cultural and technological pedigree in order to avoid major misinterpretations. For instance, certain

patients interpreted as being "multiply fruit sensitive" as a result of test feedings of commercially available fruits, have been found to be intolerant to one or more chemicals with which various fruits are treated.

In view of such observations, other chemical additives such as bread softeners, flour bleaches, sulfur dioxide treatment of foods, sodium benzoate, and other synthetic preservatives employed in food and drug manufacture should be studied in individuals prone to develop allergic type reactions from various chemical exposures.

Although chemical contaminants and preservatives of foods and drugs may be the cause of allergic type symptoms in patients intolerant to various synthetic drugs and other ingestant, injectant, inhalant, and contactant exposures to petro-, chloro- and/or sulfo- chemicals, such observations should not depreciate the common existence of allergy to chemically uncontaminated foods and pharmaceuticals of natural origin.

140. ALLERGIC TYPE REACTIONS TO SYNTHETIC DRUGS AND COSMETICS

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

Sensitivity to synthetic drugs, the most widely recognized aspect of clinical intolerance to chemicals, occurred in the following order in 80 patients having advanced degrees of this syndrome: sulfonamides, 43 cases; aspirin, 38; synthetic vitamins, 25; antihistamines, 25; phenobarbital, 24; other barbiturates, 15; local anesthetics, 14; phenophthalein, 13; and meperidine (Demerol), 11.

Intolerance to a drug may be first manifested by failure to obtain the previous degree of therapeutic relief. Then a given dose or short period of treatment may be associated with development of fatigue, transient chilling, neutropenia, parosmia, or some other slight ordinarily undetected response. Subsequently a single dose precipitates an acute reaction.

Not only may acute localized or constitutional allergic type symptoms follow exposure of highly susceptible individuals to intermittent small doses of these or other synthetic drugs or cosmetics, but if successive quantities are received while the subject is still reacting to previous ones, a chronic addictive type of response may develop. This course of events, particularly apt to occur when a patient is taking medications regularly for the "control" of his increasingly regularly recurrent symptoms, often results in chronic illness actually being perpetuated by the oft-repeated, overzealous, and ever-increasing use of such symptomatic measures. Such an overtreatment addictive type of drug response to which allergic individuals are particularly prone is exceedingly common and exists to various degrees. In several severe cases, incessant cough, extreme fatigue, headache, and generalized edema subsided for the first time in months coincident with avoidance of all medications taken for their relief—these medications being actually responsible for the perpetuation of the illnesses.

Similar acute and chronic addictive type responses, of such frequent occurrence in patients intolerant to petro-, chloro- and/or sulfo- chemicals, involve petrolatum, mineral oil, or wax bases of creams, ointments, and lotions as well as diverse synthetic colors, flavors, perfumes, and preservatives employed in the manufacture of drugs and cosmetics.

Allergic individuals, apparently increasing in number and woefully in need of recognition of currently existing sensitivities as well as protection against developing new ones, receive little of either from a medication-mad, mind-over-matter, medical mentality.

141. SODIUM BICARBONATE IN THE TREATMENT OF ALLERGIC CONDITIONS

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In keeping with the acid-anoxia-endocrine theory of allergy, sodium bicarbonate intravenously (7.5 Gm. per 100 ml.) in amounts between 3.75 and 15.0 Gm. is effective in relieving many acute and some chronic allergic manifestations. Helpful in acute rhinitis, asthma, pruritus, and urticaria, intravenous soda is particularly effective in the symptomatic control of nausea, headache, myalgia, and at least certain depressed phases of cerebral allergy. Preliminary evidence indicates that intravenous sodium bicarbonate is immediately effective in treating acute shock and some penicillin and transfusion reactions.

Intravenous administration of the alkali (at the rate of 5 to 10 ml. per minute) is stopped coincidentally with the cessation of reactive symptoms or the development of excessive thirst. In allergy patients previously diagnosed and controlled, one injection usually relieves acute reactions resulting from the inhalation of specific allergens or chemical fumes acting similarly, but injectant or ingestant reactions in which the responsible food, drug, or chemical is retained in the alimentary canal may require additional doses.

Loss of effectiveness from repeated use or other complications, except for occasional transient chills, has not been observed in the treatment of 150 cases. One patient, who develops nausea, lethargy, myalgia, incoordination, and altered consciousness following exposure to certain foods, petro-, chloro-, and sulfo- chemicals or their combustion products, has received 150 injections.

Intravenous alkalinization with sodium bicarbonate sometimes accentuates certain instances of severe protracted bronchial asthma or protracted stages of the nervous, hyperactive, ataxic phase of cerebral allergy associated with polydipsia.

Allergic reactions following the ingestion of allergenic foods or other materials acting similarly are also lessened in intensity and duration by taking 5.0 Gm. doses of 80 per cent sodium bicarbonate and 20 per cent potassium bicarbonate in water orally at half-hour intervals until the onset of symptomatic relief or the development of excessive thirst or diarrhea. When avoidance of causative agents is not feasible, the general level of chronic allergic manifestations may be decreased as a result of the daily ingestion of 2.0 to 10.0 Gm. of this alkali mixture.

Patients with impaired renal or cardiac function have not been treated.

142. A FAMILIAL TRAIT CHARACTERIZED BY DEFICIENCY OF A CLOT-PROMOTING FRACTION OF PLASMA

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Three patients have been studied in whom the clotting time of venous blood was greatly prolonged. None had significant hemorrhagic symptoms. The trait was familial and appeared in both sexes. The prolonged clotting time was the result of a defect in the plasma and not in the formed elements of blood. The abnormality in plasma was due neither to a circulating anticoagulant nor to a deficiency of known clotting factors. The thrombin time and prothrombin time were normal, suggesting that the defect resided in the *development* of thromboplastic activity in shed blood. Nevertheless, the defect could be corrected by plasma from patients with hemophilia or with de-

ficiencies of plasma thromboplastin component (Christmas factor), plasma thromboplastin antecedent, or the fourth thromboplastin component described by Spaet, Aggeler, and Kinsell. Presumably, the abnormality in thromboplastin production was due to a deficiency of some additional factor.

Such a factor could be prepared from normal serum which had been adsorbed with barium sulfate (to remove prothrombin, proconvertin, and plasma thromboplastin component) and heated at 56° C. for thirty minutes (to remove any traces of proaccelerin and antihemophilic factor). This clot-promoting fraction not only corrected the defect in the patient's plasma, but also shortened the clotting time of normal native plasma. It was virtually without effect on hemophilic native plasma. The clot-promoting fraction did not alter the rate at which fibrinogen was converted to fibrin by thrombin, nor the rate at which prothrombin was converted to thrombin by tissue thromboplastin. Thus, the clot-promoting fraction was probably concerned with an earlier step, presumably the evolution of thromboplastic activity in shed plasma.

These three patients, then, had a deficiency of a clot-promoting fraction not identifiable with known clotting factors. This fraction is apparently needed for the optimal development of plasma thromboplastic activity. Its properties may account for some of the characteristics of previously described clotting factors which may have been contaminated with it. The extraordinarily benign clinical manifestations in spite of the prolonged clotting time may explain why the trait and the factor needed for its correction have not received attention.

143. A BIO-ASSAY FOR CIRCULATING PLASMA INSULIN

CHARLES H. READ, M.D., IOWA CITY, IOWA

(INTRODUCED BY CHARLES D. MAY, M.D.)

Adult female mice were made diabetic by the intravenous injection of alloxan and subsequently were hypophysectomized. Gavage feedings of 10 per cent glucose were given at four successive hourly intervals prior to the assay in an attempt to avoid the marked metabolic lability which these delicate animals exhibit. Under this condition of prior glucose loading it was found that the fall of blood glucose proceeded at a constant rate. Thus, by determining the slope of the declining blood sugar between 45 and 105 minutes after the last gavage, it was possible to predict the blood glucose level in a further 60 minutes. Insulin-containing materials injected intraperitoneally immediately after obtaining the second blood sample were found to increase the rate of fall of the blood glucose. By this procedure each animal served as its own control.

The sensitivity of the assay was investigated and its specificity tested with sodium-l-thyroxin, pituitary growth hormone, and adrenal cortical extract. A few preliminary assays of the insulin-like activity of the plasma of normal children and an insulin-resistant juvenile diabetic will also be reported.

144. A DEFECT IN KETONE METABOLISM IN CIRRHOSIS OF THE LIVER

LILLIAN RECANT, M.D., ST. LOUIS, MO.

It is known that the liver is the major site of ketone body production and that this function is intimately related to the status of carbohydrate metabolism. Since abnormalities of carbohydrate utilization have been described in liver disease, a study of ketone metabolism in patients with cirrhosis of the liver and in animals with experimentally induced ethionine liver injury was undertaken.

The development of ketonemia in response to the stress of a standard 18-hour fast was used as an index of the ability of the liver to produce ketones. In 26 normal subjects a mean blood ketone level of 2.28 mg. per cent with a range of 0.82 to 5.20 mg. per cent was found. In 16 hospitalized patients with nonhepatic diseases, the level was 2.06 mg. per cent and the range, 0.6 to 5.4 mg. per cent. However, in 27 patients with cirrhosis, chiefly of the alcoholic variety, a significantly lower level of blood ketones was obtained, namely, 0.94 mg. per cent with a range of 0.00 to 1.30 mg. per cent. Some correlation between the state of compensation and the ketone level was observed.

The ability of orally administered glucose to reverse this starvation-induced ketonemia was studied in an effort to grossly evaluate the interrelationship between carbohydrate and fat metabolism. In 12 normal subjects, glucose reduced the ketone level by 1.07 mg. per cent in two hours; in 5 hospitalized controls by 1.40 mg. per cent; while in 15 cirrhotic subjects the ketone level fell only 0.35 mg. per cent. No abnormalities in glucose tolerance or in the levels of pyruvate and lactate were observed which could account for this difference. Fructose infusions diminished the ketonemia more strikingly than glucose in 2 out of the 3 cirrhotics studied.

Animal experiments appear to support the defect in ketone formation in liver disease. Butyrate feeding induced a ketonuria of 8.9 mg. per 100 gram rat in 24 hours, in 9 controls rats, while in 10 rats with ethionine liver damage, the ketonuria was of the order of 4.1 mg. Liver slices from these animals are currently under study to more precisely define the metabolic defect.

145. EVIDENCE FOR CONTRACTION OF THE PULMONARY INFUNDIBULAR RING AS A MODE OF STENOSIS IN MAN

SIMON RODBARD, M.D., AARON B. SHAFFER, M.D. (BY INVITATION), AND PHILIP BROSTOFF, M.D. (BY INVITATION), CHICAGO, ILL.

The timing and contour of the pressure pulses recorded in the right ventricle and infundibular chamber in cases of infundibular pulmonic stenosis provide physiologic evidence for contraction of the subinfundibular ring during systole. Normally, the pressures in the ventricle and infundibular segment begin to rise at the onset of systole; this is followed after the period of isometric contraction by a rise of similar magnitude and contour in the pulmonary arterial pressure pulse.

In the case of infundibular stenosis, the ventricular and infundibular pressures rose together at the same slope and virtually simultaneously during the period of isometric contraction, but after about 0.1 second the pressure in the infundibulum usually began to fall while the ventricular pressure continued to very high levels. Such a sequence could occur only if the subinfundibular ring was open to its maximum extent at the onset of systole, but became progres-

sively narrowed as ventricular systole proceeded. This provides dynamic evidence that, at least in part, the stenotic process in subinfundibular stenosis is due to muscular contraction.

In one case of infundibular stenosis, the pressures during systole in the infundibulum and in the main right ventricle rose together, reached their peaks simultaneously and fell together, indicating that the size of the subinfundibular ring was not decreasing during systole and, therefore, that muscular contraction was not here increasing the severity of the infundibular stenosis during ventricular systole.

The present study demonstrates that a careful time-comparison of the pressure pulses in the right ventricle and infundibulum may provide evidence concerning the physiologic or anatomic nature of the stenotic lesion.

146. ACQUIRED AGAMMAGLOBULINEMIA WITH HYPERSPLENISM

ROBERT J. ROHN, M.D., ROY H. BEHNKE, M.D. (BY INVITATION), AND WILLIAM H. BOND, M.D. (BY INVITATION), INDIANAPOLIS, IND.

In the past several years there has been increasing interest in the clinical syndrome associated with plasma agammaglobulinemia and hypogammaglobulinemia. The majority of these reports have dealt with this entity in children, and with rare exceptions this has been regarded as a hereditary disorder. This case occurred in a 29-year-old white man and seems, on the basis of the accumulated data, to represent an acquired agammaglobulinemia. Of additional interest, a hypersplenic mechanism was present which was corrected by splenectomy.

The case is presented with the following substantiating laboratory and clinical data: serial Tiselius and paper electrophoretic curves, showing the utilization of specific, intramuscularly injected gamma globulin; serial peripheral blood studies before and after splenectomy; negative skin tests; absence of demonstrable antibodies; photomicrographic studies of splenic and lymphoid tissue showing fibrosis, lymphopenia, and reticulum cell hyperplasia; bacteriologic studies; paper electrophoretic and hematological studies on other members of the family.

It would seem logical to assume, in the absence of demonstrable involvement of other members of the family, that this is a case of acquired agammaglobulinemia. The findings in this patient would seem to support two additional postulates: (1) As no antibodies could be demonstrated, and in the absence of gamma globulin, it would seem that hypersplenism may in some instances be entirely a result of hyperphagocytosis by the reticulum cell elements of the spleen. (2) It would seem, from the observations in this patient, with increased reticulum cells and clasmatocytes showing hyperactive phagocytosis but marked decrease in lymphocytes, that antibody formation may be a specific function of the lymphocyte.

The pathogenesis of the profound disturbance in the reticuloendothelial tissues of this patient is presently a matter of speculation.

147. C^{14}-ACETATE UTILIZATION IN DIETARY NECROSIS OF THE LIVER

MARVIN ROSECAN, M.D., GERALD P. RODNAM, M.D., SIDNEY S. CHERNICK, PH.D., AND KLAUS SCHWARZ, M.D., BETHESDA, MD.

(INTRODUCED BY ROBERT E. SHANK, M.D.)

Dietary hepatic necrosis is induced in rats by feeding a ration containing Torula yeast as the sole source of protein. The diet is free of vitamin E, and deficient in cystine and Factor 3. The combined deficiency is characterized by the sudden occurrence of fatal acute hepatic necrosis after approximately 45 days.

In a previous report (Fed. Proc. 13: 191, 1954) a characteristic metabolic defect in the livers of rats fed this diet was described. Liver slices from these animals were found to respire at normal rates for 30 to 60 minutes; then respiration failed as in vitro incubation proceeded. Slices were taken from areas of the liver which were demonstrated to be free of degenerative changes by gross and histologic examination. Kidney slices and diaphragms from deficient rats respired normally for two hours. This alteration in oxidative metabolism was not produced by other agents known to induce hepatic damage. Supplementation of the Torula diet with vitamin E prevented the respiratory defect and the development of hepatic necrosis.

The original observations have been extended with the investigation of three metabolic pathways of 2,C^{14}-acetate—ketogenesis, lipogenesis, and oxidation. Total blood ketones of animals fed the Torula diet, prior to fasting for 42 and 56 hours, were lower than those of similarly treated rats receiving vitamin E. Livers of the animals were characterized, in vitro, by progressive failure of utilization of acetate for lipogenesis, ketogenesis, and oxidation to CO_2. The defects differ from those observed in fasting normal rats and in diabetic animals. Under these latter conditions the liver retains the capacity to oxidize acetate to CO_2 and to form ketones.

Supplementation of the Torula diet with vitamin E, or intraportal injection of an emulsion of vitamin E, restored respiration and C^{14}-acetate utilization to normal. Coenzyme A activity was not low in these deficient livers. It is suggested that in this form of dietary hepatic necrosis the defect in acetate metabolism is secondary to alterations in oxidative metabolism. The biochemical defects precede frank necrosis and are prevented or corrected by administration of vitamin E.

148. HISTAMINE AS THE POSSIBLE CHEMICAL MEDIATOR FOR CUTANEOUS PAIN (DIRECT AND REFERRED)

SOL ROY ROSENTHAL, M.D., PH.D., CHICAGO, ILL.

Experimental evidence in animals and man is presented to demonstrate that electrical, physical, or chemical stimuli applied to skin (or cornea in animals) cause it to liberate a histamine-like substance in direct proportion to the degree of stimulation. That this substance is most likely histamine was demonstrated by usual biologic methods plus the fact that certain of its actions are neutralized by antihistamine drugs. When injected intracutaneously into human subjects, it produces local reactions and a pattern of painful response similar to that of histamine. The pain has an immediate and latent component. Its intensity and duration are directly related to the concentration of histamine-like substance. Painful sensations may be produced by the injection of

histamine intradermally in concentrations of 10^{-18} (not augmented by acetylcholine or adenosine). The method employed was to obtain split thicknesses of skin (electrically driven dermatome) and to secure them over one open end of glass cylinders with or without interposing cellophane membranes. By placing saline within the glass cylinder, the products resulting from stimulation of the skin are allowed to perfuse into the saline (facilitated by electrodialysis). Indirect evidence that histamine is the possible chemical mediator for cutaneous pain was demonstrated by showing that antihistamine drugs as well as histamine analogues act as local anesthetics.

Abdominal pain is referred to the skin and is mediated by histamine since: (a) Stimulation of a greater splanchnic nerve (dogs) liberates histamine in corresponding skin of abdomen; (b) subcutaneous injection on antihistamine thymoxyethyldiethylamine (25 mg. per kilogram) produces generalized local anesthesia without suppressing the higher centers. In such animals, electrical stimulation of the splanchnic or mesenteric nerves or visceral or parietal peritoneum fails to evince pain in the dog. However, the threshold of saphenous nerve remains unchanged.

149. THE HEART IN MUSCULAR DYSTROPHY

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Several reviews on the subject of muscular dystrophy have indicated that the heart is involved in a high percentage of patients with this disease. Apart from the autopsy findings, the major basis for this deduction was the apparent frequency of electrocardiographic abnormalities. Critical review of the electrocardiograms presented in these articles resulted in detecting few impressive findings that could not be considered as representing normal physiological variation or coincidental cardiac disease.

This report consists of a detailed clinical and electrocardiographic analysis of thirty-three patients with muscular dystrophy. Three of the patients, including two with hypertension and one with an anteroseptal myocardial infarction, presented expected electrocardiographic abnormalities. Of the other thirty patients three presented abnormal ventricular complexes and two of these were suggestive of right ventricular enlargement.

The previously described abnormalities of tall R waves in Leads V_1 and V_2, abnormal Q waves, and similarities of leads aVR and aVL, were in most instances in our series simply associated with the vertical heart position and clockwise rotation in occasional combination with backward rotation of the apex positions seen in normal young patients of this age group and in those with the chest deformities encountered in muscular dystrophy. The occurrence of arrhythmias, sinus tachycardia, short P-R intervals, and abnormal P waves which were mentioned in the previous studies were not abnormally seen in our group of cases for only three instances of sinus tachycardia and one of auricular extrasystoles were found. The only cardiac complication of the entire series occurred in a 15-year-old boy with a normal electrocardiogram. Following an upper respiratory infection, this patient developed atelectasis with right ventricular dilatation and cardiac failure with subsequent slow improvement on therapy.

Ballistocardiograms could not be taken due to the inability of the patients to relax the muscle groups sufficiently for the test. No specific electrocardiographic pattern was found to be indicative of dystrophic involvement of the heart. This diagnosis must be based primarily upon clinical suspicion and the subsequent course of the disease.

150. CORONARY BLOOD FLOW, CARDIAC WORK, AND MYOCARDIAL METABOLISM IN THYROTOXICOSIS BEFORE AND AFTER TREATMENT

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Ten patients with thyrotoxicosis ranging from 11 to 74 years of age have been studied by cardiac catheterization with determination of cardiac output by the Fick principle and coronary blood flow by the nitrous oxide method. These patients demonstrated an increase in oxygen consumption, cardiac rate, cardiac output, left ventricular work, coronary blood flow, and cardiac oxygen utilization. Those patients studied again after treatment with surgery or radioactive iodine revealed a significant decrease in each of these functions. The coronary vascular resistance which was reduced initially was increased after treatment. When the cardiac oxygen utilization was related to cardiac rate, the oxygen consumption per beat remained unchanged with treatment.

These findings indicate that the coronary vascular response and myocardial metabolic changes are similar to those seen in other tissues in the presence of excessive thyroid hormone.

151. THE URINARY PORPHYRIN TEST IN DETECTION OF LEAD ABSORPTION

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The erythrocyte basophilic stipple count has been proved to be a valuable laboratory aid in detecting lead absorption. More recently the detection of urinary porphyrins, a simple procedure, has been advocated as a more sensitive screening test.

In comparing the merits of the stipple count and the urinary porphyrin test, it was demonstrated that the ultraviolet light source was a most important variable in the results obtained with the porphyrin test. Employing a mercury lamp with a blue-purple ultra filter and a core and coil ballast and using the method of McCord slightly modified, comparisons were made with the stipple count in 67 consecutive tests on battery factory workers.

In these 67 tests, there were 29 which showed stippled cells and 45 positive urinary porphyrin tests. There were 4 instances where stippled cells were seen accompanied by negative porphyrin tests. On the other hand, there were 21 positive porphyrin tests when no stippled cells were present.

From these figures, it seems probable that the detection of urinary porphyrins, employing a suitable ultraviolet light source, is a more sensitive procedure than the count of stippled erythrocytes in the detection of lead absorption.

152. THE INFLUENCE OF ADVANCED CONGESTIVE HEART FAILURE ON PULSUS ALTERNANS

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(INTRODUCED BY B. K. WISEMAN, M.D.)

Recent observations indicate that, once established, pulsus alternans may be profoundly influenced by changes in the venous return to the heart. Increasing this return appears to diminish while decreasing it tends to exaggerate alternation.

We have observed twelve patients with pulsus alternans during the past two years. Three showed the virtual disappearance of their alternans with severe congestive heart failure. One of these three was in intractable failure and never demonstrated a return of his alternans, even after prolonged motionless standing. The remaining two showed a reappearance of their alternans as they responded satisfactorily to treatment. The remaining nine were never in advanced congestive failure and have shown persistent pulsus alternans. All nine were being treated in the usual manner for cardiac failure at the time of the observations.

It is suggested that with severe congestive heart failure the ventricular filling pressure has increased to the extent that the diastolic stretch of the left ventricle becomes more equal from cycle to cycle. The resulting ventricular responses are more uniform and thus alternation diminishes. This situation is somewhat similar to that found when pulsus alternans is decreased with the increased venous return of exercise, passive leg raising, or infusions.

Pulsus alternans usually indicates myocardial insufficiency and is therefore of clinical value. These observations would seem to indicate that, in addition, the disappearance of this phenomenon in the face of progressive congestive failure may also be of clinical importance. They further suggest that pulsus alternans might be more easily detected not only when the patient is upright but also as he responds to treatment for congestive failure.

153. SOME MATHEMATICAL CONSIDERATIONS OF THE VOLUME OF DISTRIBUTION OF RADIOSULFATE

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(INTRODUCED BY FORD K. HICK, M.D.)

Walser, in 1952, showed that within 15 to 20 minutes following a single intravenous injection, radiosulfate reached an equilibrium between the vascular and interstitial spaces. He also showed that the calculated volume of distribution of radiosulfate increased with time and that this increase could not be accounted for by urinary or fecal excretion. He proposed that the 18-minute radiosulfate space could be used as an index of extracellular fluid volume.

Because of the expanding volume of distribution it is theoretically more correct to calculate a zero time volume of distribution. The classical method for calculating a zero time space is to divide the administered dose by the zero time plasma concentration. This method assumes, however, that the loss of material from the space to be measured is uniform from the moment of injection. Such is not the case with radiosulfate. Radiosulfate disappears from

the body by urinary excretion at two exponential rates. The first rate is rapid, lasts twenty minutes, and is the result of higher plasma than interstitial fluid concentrations during the equilibrium period. The second rate is slower and uniform for nine hours after injection.

Due to the "excessive" urinary loss during the equilibrium period, part of the injected radiosulfate never becomes distributed in the space to be measured and hence does not contribute to the zero time plasma concentration. This "excessive" loss may be corrected by extrapolating the second slower rate of disappearance from the body to zero time. The zero time amount of radiosulfate remaining in the body is looked upon as the "effective administered dose" divided by the zero time plasma concentration.

We have applied some of the hypotheses derived by Best in his work with fluid analogs in an attempt to elucidate the expanding volume of distribution of radiosulfate. If one assumes that the zero time radiosulfate space remains constant during the test period, then the amount of radioactivity in the space at any given time is the product of the zero time volume and the concentration at that given time. The amount of radioactivity remaining in the body at a given time is measured (administered dose minus cumulative urinary excretion). The nonurinary loss of radiosulfate is the difference between that remaining in the body and that remaining in the zero time radiosulfate space. The nonurinary loss is found to increase to a maximum and then decrease. We then made the assumption that the nonurinary loss was due to penetration of radiosulfate into a "second" fluid compartment in diffusion equilibrium with the zero time space. If this is true at the time of maximum loss (no net exchange between the two spaces), the concentrations in the two compartments are equal. Thus knowing the total radioactivity and the concentration of radioactivity, the volume of the "second" space may be calculated.

To test these hypotheses the volume of distribution of radiosulfate and thiocyanate was determined simultaneously on eleven young soldiers on two occasions. The zero time radiosulfate and thiocyanate spaces were 19.1 (SD ± 1.7) and 25.6 (SD ± 2.0) per cent of body weight, respectively, on the first occasion. They were 18.6 (SD ± 1.6) and 27.4 (SD ± 3.5) per cent of body weight on the second occasion. The volume of the "second" radiosulfate space was not found to be reproducible entity, correlate with the difference between the zero time radiosulfate and thiocyanate spaces, or conform to any known fluid compartment. It is, therefore, concluded that the nonurinary loss of radiosulfate is not the result of simple diffusion into a fluid compartment in equilibrium with the zero time radiosulfate space.

The concept of an "effective administered dose" may be applied to the calculation of zero time volume of distribution of mannitol and sucrose using the single injection technique. The concepts used in calculating the "second" tracer space, although not particularly helpful with radiosulfate, may be of value in explaining some phenomena of other tracer substances.

154. THE ZERO TIME RADIOSULFATE SPACE IN NORMAL AND ABNORMAL SUBJECTS

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CHICAGO, ILL.

(INTRODUCED BY FORD K. HICK, M.D.)

The zero time volume of distribution of radiosulfate, following a single intravenous injection, was determined on 22 normal subjects. In nine young men it was found to be 16.7 (SD ± 2.2) per cent of body weight; in five elderly

men it was 14.9 (SD ± 3.2) per cent of body weight, while in eight elderly women it was 15.5 (SD ± 2.1) per cent of body weight. The differences between these groups were not statistically significant.

Four subjects had simultaneous radiosulfate and inulin space determinations. Inulin space was measured by the five-hour infusion technique. These spaces were found to agree closely. The mean radiosulfate-inulin space ratio was 1.05.

Five subjects had a second determination of their radiosulfate space one to thirty days following the initial measurement. The space was found to be quite reproducible. The mean difference between the first and second determinations was −0.12 liter or −0.04 per cent of body weight.

Four abnormal subjects were studied. In one patient with diabetic acidosis and moderate dehydration the zero time radiosulfate space was 10.5 per cent of body weight. In a severely dehydrated subject who had suffered a cerebrovascular accident it was 7.2 per cent of body weight. In two patients with ascites the zero time space was 26.2 and 22.8 per cent of body weight. A five-hour equilibrium period was found to be necessary in these subjects with abnormal collections of fluid.

The zero time radiosulfate space meets the following criteria for use as an index of extracellular space: (a) a safe and technically feasible method; (b) reproducibility in the steady state; (c) variation in the clinically indicated direction and degree in subjects with abnormalities of the extracellular space; (d) correlation with other indices of the extracellular space; (e) a rapid and uniform distribution in the extracellular fluid; (f) a relatively slow exponential loss from the extracellular space.

155. EFFECT OF HEAD-DOWN BODY TILT AND HEXAMETHONIUM (C6) IN PATIENTS WITH CHRONIC PULMONARY EMPHYSEMATOUS HYPERTENSION

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(INTRODUCED BY ROY W. SCOTT, M.D.)

The effect of C6 on the pulmonary dynamics during horizontal positioning and following a period of stabilized head-down tilt has been studied by cardiac catheterization in 14 steady state patients with uncomplicated chronic obstructive pulmonary emphysematous hypertension. Observations on normal individuals served as controls.

C6 given in the maintained horizontal position produces a significant decrease in total pulmonary resistance (PuTR) in normal subjects, and no changes in emphysematous patients ($p = 0.01$). Stabilized tilting produces significant and comparable increases of PuTR in both groups, but pulmonary "arteriolar" resistance (PuArR) decreases in normal and remains unchanged in emphysematous patients ($p = 0.001$). Administration of C6 during tilt reduces PuTR and increases PuArR to pretilt levels in the normal subject, but causes no change from the tilt-induced values in the emphysematous subject.

The data confirm the concept of relative indistensibility of the small artery bed in the slightly hypoxic pulmonary hypertensive emphysematous patient, and adduce no evidence that a direct extrinsic sympathetic neurogenic mechanism plays a role during the steady resting state in the maintenance of this type of hypertension.

Further evidence is presented to demonstrate that autonomic "blocking agents" are dangerous in subjects with intrinsic pulmonary hypertension.

156. STUDIES WITH ETHYL VANILLATE IN EXPERIMENTAL HISTOPLASMOSIS

SAMUEL SASLAW, M.D., PH.D., AND ROBERT MACMILLAN, A.B., M.S. (BY INVITATION), COLUMBUS, OHIO

Ethyl vanillate has been reported as showing promise in human histoplasmosis. However, to our knowledge there have been no published data concerning its effect in experimental infections in animals.

White Swiss mice infected with yeast phase *H. capsulatum* were treated with ethyl vanillate, by gavage, in doses varying from 0.01 to 0.1 Gm. daily or twice daily for thirty days or less. In one group the drug was diluted in cottonseed oil, and in the other Tween 80 and distilled water were the diluents. Infected controls received the menstruum only. No difference in mortality was noted between the treated and untreated mice. Since the sera of treated mice demonstrated adequate levels of the drug, one could not attribute the lack of effect to nonabsorption. Preliminary studies in dogs have also demonstrated no therapeutic effect. Although these studies do not exclude the possibility that ethyl vanillate may be effective in human histoplasmosis, one must be cautious in evaluating therapeutic results in such a protean disease.

157. STUDIES ON PROLONGED ADMINISTRATION OF CHLOROMYCETIN IN MONKEYS

SAMUEL SASLAW, M.D., PH.D., CHARLES A. DOAN, M.D., AND RICHARD L. SCHAFER, M.D. (BY INVITATION), COLUMBUS, OHIO

For the past two years an attempt has been made to produce bone-marrow depression in forty-three rhesus monkeys by administration of Chloromycetin.

Varied regimes were employed with dosages as high as 5 Gm. daily for over a year. Continued and intermittent regimes were employed in different groups of monkeys at various dose levels. Monkeys previously bled to anemic levels as well as those receiving total body irradiation or with nutritional cytopenias were administered Chloromycetin. In no instance was it possible under these varying conditions to induce bone-marrow depression in normal monkeys or prevent the return to normal in those animals previously insulted with irradiation, bleeding, or diet.

158. ADULT AGAMMAGLOBULINEMIA

SAMUEL SASLAW, M.D., PH.D., AND ROBERT L. WALL, M.D. (BY INVITATION), COLUMBUS, OHIO

Agammaglobulinemia, previously described in children, has been observed to occur in two adults. One 26-year-old man with mental retardation and multiple congenital defects experienced repeated episodes of bacterial pneumonia.

This patient had a deficiency in gamma globulin and was unable to form antibodies after immunizing doses of triple typhoid, influenza, and mumps vaccines. Following the latter vaccine his skin test for mumps was negative. He was also Shick positive and tuberculin and histoplasmin negative. The second patient, a 40-year-old man, had a three-year history of repeated infections associated with a spruelike syndrome. He likewise had no gamma globulin and failed to form antibodies after immunization with triple-typhoid vac-

cine. Both patients had otherwise normal serum protein patterns. With the more widespread application of paper-strip electrophoresis and antibiotics, it is suggested that more adults with this syndrome will be detected in the future.

159. PROTEINS AND CARBOHYDRATES IN SERUM IN ACUTE VIRAL HEPATITIS DETERMINED BY PAPER ELECTROPHORESIS

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(Introduced by Hans Popper, M.D.)

In 13 patients with acute viral hepatitis, blood was taken at weekly intervals from the first week of onset of the disease until convalescence was complete. In addition to the usual hepatic tests, serum polysaccharides, protein-bound polysaccharides, and serum mucoproteins were determined chemically and serum protein fractions and glycoprotein distribution in these fractions were determined densitometrically after paper electrophoresis. The results were compared with those in 12 normal controls at weekly intervals. The results showed that by the time the patient reached the hospital, usually the third day of jaundice, the alpha$_1$ globulin had dropped to below the lower limit of normal in all but two cases which became low the following week. Gamma globulin had risen by the time of admission and the peak rise was attained by the second week in ten cases while the lowest alpha$_1$ values were not reached until the fourth week or later in six cases. Albumin values moved in an opposite direction to gamma globulin. The other protein fractions showed no consistent behavior. During convalescence, gamma globulin returned to normal or almost normal before alpha$_1$ globulin, and, in general, the results of the hepatic tests were normal before the proteins. No consistent changes were found in the results of chemical determinations of serum polysaccharides or mucoproteins (determined by an orcinol method and by a turbidimetric precipitation method). However, carbohydrates migrating with protein appeared to shift toward albumin and alpha$_1$ globulin away from gamma and beta fractions early in the disease and slowly returned toward normal with convalescence, in spite of changes in the relative protein values in the opposite direction.

160. THE ACUTE EFFECTS OF INTRAVENOUS INJECTION OF TWEEN 60 FAT EMULSIONS

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Previous experience with the injection of 0.5 per cent solutions of Tween 60 (polyoxyethylene sorbitan monostearate) in animal experiments (J. L. Schmidt, E. L. Goodsell, and R. K. Richards, J. P. E. T. **106**: 413, 1952) indicated that these solutions deserved study in man.

A small series of volunteers and patients received intravenous infusions of up to 500 ml. of 0.5 per cent Tween 60 in 5 per cent dextrose without event except in one instance of a terminal case on the brink of cardiac decompensation. Pulse, blood pressure, electrocardiograms, and, in one instance, a ballistocardiogram were recorded.

Fat emulsions containing 0.5 per cent Tween 60 were well tolerated in rabbits and monkeys. The cat, which tolerates the intravenous injection of 0.5 per cent Tween 60 quite well, responded with a precipitous fall of blood pressure, bradycardia, and apnea following the injection of a few milliliters of these emulsions. Recovery occurred within a few minutes and a second injection of the Tween 60 fat emulsion was without effects. Similar falls in cat blood pressure were observed following the injection of fat emulsions prepared with lecithin.

Cautious trials of Tween 60 fat emulsions were then made in volunteers and patients. Four individuals were studied, three of whom experienced flushing of the face, epigastric or substernal distress, and cough after only a few milliliters of the emulsion had been injected. After these initial symptoms subsided, the infusion was continued in one individual for a total of 50 ml. without further signs or symptoms.

In the cat heart-lung preparation the administration of large doses of the Tween 60 fat emulsion led to cardiac failure. In the intact "Nembutalized" cat transection of the vagi prevented the apnea and a rise in blood pressure frequently occurred. However, doses of procaine sufficient to block serotonin apnea and hypotension were ineffective against the effects of the Tween 60 fat emulsions in similar preparations.

In open chest experiments or following pneumothorax, the effects following injection of Tween 60 fat emulsions were largely abolished.

Electrocardiographic changes, comparisons with the effects of lecithin fat emulsions, and the implications of the experiments, will be discussed.

161. ODOR PERCEPTION THRESHOLDS FOR "CITRAL" UTILIZING A NEW TYPE OLFACTORIUM

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With the technical assistance of Bernard Dolenz, B.S. (By Invitation), and James Williams, B.S. (By Invitation)

A new type odor-free, temperature and humidity controlled olfactorium was utilized in 140 perception threshold measurements for "citral" in 32 female and 14 male healthy subjects, aged 18 to 45 years. The test room (485 cu. ft.) and adjacent air-lock, with airtight doors, were fully lined with aluminum foil to minimize air leakage and odor absorption. A recirculating air conditioner and humidifier with a battery of carbon filter deodorizers in the return duct supplied odor-free air at 70° F. and 50 per cent humidity. The tip of an ultramicroburette projected into the test room and allowed the accurate introduction of "citral" (increments of 0.0001 ml.) which was vaporized by an atomizing jet of deodorized, dry air at constant pressure. Thorough mixing with the test-room air was accomplished by three fans mounted within the olfactorium. Mouth breathing and gentle blockage of the nares avoided sampling of the air during the short period of odor introduction and mixing. Varying increments of citral were introduced step-wise in increasing and on other occasions decreasing amounts or at random till a standardized "sniff" allowed subjective recognition of citral. The room was deodorized and reconditioned for eight minutes between each stimulus.

Results.—(1) Female subjects had lower mean thresholds than males (excluding initial trials, subjects in the menstrual period or with nasal obstruction, mean value for females = 0.000017 mg. citral/L. air; for males = 0.000037

mg./L. air). Sex difference was highly significant, P = .01. (2) Nasal obstruction raised the mean threshold (females = 0.000048 mg./L. air, compared to females without obstruction, P = .01; males = 0.000057 mg./L. air, compared with rest of male subjects, P = .01). (3) Thresholds were significantly elevated (P = .01) during menstrual periods, mean = 0.000047 mg./L. air, and only three of these ten subjects had nasal obstruction. (4) Current situational stress was accompanied by elevated thresholds (P = .01) over that observed in nonstressful circumstances (females = 0.000034 mg./L. air; males = 0.000047 mg./L. air). (5) Subjects tested within three hours of the last meal had significantly higher thresholds (P = .01) than "hungry" subjects (three to six hours post cibum).

162. THE LONG-TERM EVALUATION OF PARENTERAL VITAMIN B_{12} AND ORAL VITAMIN B_{12} WITH FOLIC ACID IN THE TREATMENT OF PERNICIOUS ANEMIA

STEVEN O. SCHWARTZ, M.D., IRVING A. FRIEDMAN, M.D. (BY INVITATION), AND HELEN L. GANT (BY INVITATION), CHICAGO, ILL.

Since the natural history of pernicious anemia is characterized by spontaneous exacerbation and remission, long-term studies are necessary to evaluate new therapeutic agents. Because of this a study was begun over four years ago to compare the course of 32 patients with pernicious anemia treated with parenteral vitamin B_{12}, 36 patients with a combination of vitamin B_{12} (25 μg) and folic acid (1.67 mg.) by mouth, and 34 patients treated with parenteral liver extract. The course requirements, as well as the neurological and hematological status of these patients, were well established before the beginning of the study, the patients having been observed for periods varying from several months to sixteen years.

In the liver extract treated group, only three patients had slight recurrence of paresthesias with monthly injections of 30 U.S.P. units of liver extract. In the vitamin B_{12} treated group there were four neurological relapses, consisting primarily of decreased vibration sensation. These occurred in patients who were receiving 30 μg monthly. Both increased amounts of vitamin B_{12} and adequate liver therapy corrected the neurological abnormalities. In the vitamin B_{12}-folic acid treated group there was one hematological relapse (19 months), nine neurological relapses (15 to 35 months), and one combined neurological and hematological relapse (19 months).

In summary: (a.) Parenteral vitamin B_{12} is an adequate therapeutic agent for the treatment of pernicious anemia. (b.) Vitamin B_{12} in an average dose of 1 μg per day maintains most patients in adequate remission. (c.) Patients relapsing neurologically while receiving vitamin B_{12} may be induced to remit by the exhibition of either larger doses or more frequent administration of vitamin B_{12}. (d.) The factors of rapid excretion and the consequent depletion of vitamin B_{12} between doses are considered the prime causes of relapse. (e.) A more slowly absorbed and consequently less rapidly eliminated vitamin B_{12} should obviate these objections. (f.) Daily oral administration of 25 μg vitamin B_{12} with 1.67 mg. folic acid is inadequate for the maintenance of the patient with pernicious anemia.

163. OBSERVATIONS ON THE EFFECT OF TETRAETHYLAMMONIUM CHLORIDE ON THE PULMONARY VASCULAR RESISTANCE IN MITRAL STENOSIS

RALPH C. SCOTT, M.D., SAMUEL KAPLAN, M.D. (BY INVITATION), AND WILLIAM J. STILES (BY INVITATION), CINCINNATI, OHIO

Patients with mitral stenosis usually have an elevated pulmonary vascular resistance. The exact manner in which this increase in pulmonary arteriolar resistance is brought about is obscure.

If the increase in pulmonary arteriolar resistance is presumed to be due to both vasoconstriction and organic vascular changes in the precapillary arterioles, release of the vasomotor control should permit the assessment of what portion of the increased resistance is due to the organic changes. Such a method of predicting preoperatively if the arteriolar resistance is reversible is highly desirable. By the use of an autonomic blocking agent such as tetraethylammonium chloride (TEAC) the neural component of the increased vascular resistance should be largely inhibited. Then those cases whose arteriolar resistance remain high (and not due to a significant change in cardiac output) may be the ones with advanced organic arteriolar alterations.

The present report is concerned with the hemodynamic findings in six patients with mitral stenosis who underwent cardiac catheterization and were given 5 mg. of TEAC per kilogram of body weight.

The administration of TEAC produced a significant lowering of mean pulmonary artery pressure in five of six cases. This was due to a decrease in cardiac output in two cases and to a decline in pulmonary arteriolar resistance in three.

Two of these patients were also studied after mitral valvulotomy. The mean pulmonary artery pressure and the pulmonary arteriolar resistance were found to be lowered in both patients after surgery (to a value lower than that obtained with TEAC before surgery). The pulmonary artery pressure and pulmonary arteriolar resistance fell even further in both patients after TEAC.

These results suggest that at least in some cases of mitral stenosis a portion of the increase in pulmonary arteriolar resistance is mediated through the autonomic nervous system.

The present study is too limited in scope to draw any definitive conclusions. It is suggested, however, that a significant fall in pulmonary artery pressure secondary to a decline in pulmonary arteriolar resistance after TEAC administration in a patient with mitral stenosis preoperatively is a good prognostic sign.

164. THE TREATMENT OF ADRENAL HYPERPLASIA WITH VIRILISM BY INFREQUENT INJECTIONS OF HYDROCORTISONE ACETATE

ALBERT SEGALOFF, M.D., DOUGLAS GORDON, M.D. (BY INVITATION), ALBERT FLORES (BY INVITATION), AND B. N. HORWITT, PH.D. (BY INVITATION), NEW ORLEANS, LA.

The efficacy of cortisone therapy in the treatment of adrenal hyperplasia with virilism is well established. It has been our experience and that of others that oral therapy may be inconvenient as well as disadvantageous in many instances. Intramuscular cortisone acetate therapy has not been successfully spaced at intervals greater than four days. Therefore, in an effort to find an effective, long-acting material requiring less frequent intramuscular injections, a series of esters of cortical hormones were compared for the longevity of their

ability to maintain life in the adrenalectomized rat. It was found that hydrocortisone acetate showed the most substantial prolongation of action of any esters tested to date. Accordingly, intramuscular hydrocortisone acetate therapy was selected for trial in human beings. Coupled with its longevity of action is its slow absorption, making it necessary that substantial amounts of material be injected to attain threshold absorption.

Five females with virilizing adrenal hyperplasia have been treated with this material. Each showed a satisfactory laboratory and clinical remission with a minimum dose of 250 mg. intramuscularly once weekly. The following therapeutic regime seems to be most favorable: 250 mg. of hydrocortisone acetate is given intramuscularly once weekly until a constant excretion of both 17-ketosteroids and pregnanediol chromogens at an adequately low level is obtained, associated with clinical remission. After this occurs, the interval between injections may be gradually lengthened. In one instance it has been possible to space each injection every three weeks. In this instance, however, 500 mg. of hydrocortisone acetate are required at each injection to maintain adequate laboratory and clinical remission.

Our experience with this therapy has been so favorable that it can be suggested that infrequent intramuscular injections of hydrocortisone acetate would probably be a method of choice for treatment of patients with Addison's disease or other instances where low dosage cortical hormone therapy is required.

165. THE EFFECT OF LARGE DOSES OF SYNTHETIC VITAMIN K AND K_1 ON THE PROTHROMBIN TIME OF PATIENTS WITH LIVER DISEASE

HAROLD SHRIFTER, M.D. (BY INVITATION), AND FREDERICK STEIGMANN, M.D., CHICAGO, ILL.

Some workers reported that large amounts of antihemorrhagic substances (synthetic vitamin K) administered to patients with liver disease not only failed to restore prolonged prothrombin time to normal but actually increased it temporarily in some instances. These changes were observed almost exclusively in the prothrombin time of diluted (12.5 per cent) plasma.

Because of the above, recent introduction of Mephyton, an emulsion of vitamin K_1, was considered an aid to the clinician in improving hypoprothrombinemia in patients with liver disease.

We studied in a few liver cases with marked hypothrombinemia the results of synthetic vitamin K (Hykinone) and Mephyton. The effects of the two were compared in some of them.

Following 72 mg. of Hykinone there was an initial (first 24 hours) improvement of the prothrombin time in 11; in 3 this persisted for 48 hours. In one the prothrombin time first increased but decreased during the following 24 hours; in one the decrease was persistent; in one it remained unchanged during 48 hours.

Following 50 mg. of Mephyton there was an improvement initially in 8; with persistence for 48 hours in 4 of them. An increase in the prothrombin time occurred in one.

In 6 patients in whom the effects were compared, they were equal in 4, while in 2 Mephyton was slightly superior. In no case did the prothrombin time return completely to normal (100 per cent).

These observations indicate that large doses of synthetic K caused improvement in the prothrombin time in 12 of 14 patients, with worsening in only one case. After Mephyton there was improvement in 8 of 9 cases and worsening in one case.

Hypoprothrombinemia did not increase significantly in any patient following large doses of synthetic vitamin K. Subjectively and objectively, as measured by physical and laboratory findings, no untoward results were noted. No apparent superiority of Mephyton was noted in this preliminary study.

166. A STUDY OF DIABETES INSIPIDUS AND PRIMARY ADRENAL CORTICAL FAILURE IN THE SAME PATIENT

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This report is the first in the literature of a patient having concurrent primary failures of the posterior hypophysis and of the adrenal cortex with resultant coincidence of diabetes insipidus and Addison's disease.

The diagnosis of Addison's disease in this 51-year-old woman was based on the symptoms of nausea, anorexia, weight loss, weakness, and pigmentation, positive water and ACTH tests, low 17-ketosteroid output, normal menstrual function, and normal urinary gonadotropin titers. Cortisone relieved these symptoms. The diagnosis of diabetes insipidus was based on the output of large volumes of dilute urine, a positive (diuretic) response to intravenous hypertonic saline solution, and a prompt response to posterior pituitary extract.

An unusual clinical observation before treatment was the persistence of the delayed diuretic response to a water load in spite of the presence of untreated diabetes insipidus. Treatment with cortisone greatly augmented the over-all water diuresis and restored to normal the time of onset of water diuresis after the extra water load. Further studies during administration of cortisone indicated an increased glomerular filtration rate and decreased chloruresis.

Observations of the relationships between urine flow and osmotic load in the hydropenic state during osmotic diuresis demonstrated a water reabsorption defect of approximately 10 ml. per minute, which was not affected by a cortisone supplement but which was abolished by an infusion of Pitressin. The cortisone increased the renal plasma flow and the glomerular filtration rate; renal plasma flow diminished during infusion of Pitressin, while filtration rate was little affected.

These data characterize the abnormality of water function in diabetes insipidus and clarify some of the relationships among posterior hypophysial, adrenal cortical, and renal water functions.

167. THE DETERMINATION OF RHEUMATIC FEVER ACTIVITY BY THE TECHNIQUES OF HEMAGGLUTINATION AND HEMOLYSIS

ROBERT SKILLMAN, M.D. (BY INVITATION), WILMA SPURRIER, M.T. (A.S.C.P.) (BY INVITATION), IRVING A. FRIEDMAN, M.D. (BY INVITATION), AND STEVEN O. SCHWARTZ, M.D., CHICAGO, ILL.

The principle of the antigen antibody reaction has been used as the basis for numerous tests to identify infectious diseases. It has also been shown that erythrocytes which have been coated with an antigen are agglutinated when antisera prepared from this coating antigen is added. When complement is added to this system, hemolysis occurs under certain conditions. Since rheumatic fever is associated with tissue reaction to Type A beta hemolytic strepto-

coccus, an attempt was made to determine whether a means of identifying the specific bacterial antigen, or the antibodies elicited by it, could be used as a means of ascertaining rheumatic fever activity.

Two methods of determination were employed. In one, serum of rheumatic fever patients was used as the antibody source, and sheep red cells coated with heat-killed Type A beta hemolytic streptococcus, which had been isolated from active rheumatic fever patients, were used as the antigen source, with complement added to produce hemolysis. The other method utilized Type A beta hemolytic streptococcus antiserum obtained from rabbits, and erythrocytes of rheumatic fever patients in an agglutination test. Treating the erythrocytes with papain was found to enhance the reaction markedly in both methods.

Eighty rheumatic fever patients were tested, many at frequent intervals. The test titers by both methods became elevated within the first few days of activity in most cases, then gradually subsided and disappeared as the process became inactive. Excellent correlation was found between the results of the tests and the clinical stage of the disease, although the *height* of the titer could not be correlated with the severity of the process.

Control studies, including patients from whom alpha and beta hemolytic streptococcus was isolated, failed to reveal instances where other diseases gave positive tests.

The techniques of hemagglutination or hemolysis as herein described are of practical value in ascertaining activity or quiescence of rheumatic fever.

168. THE TREATMENT OF POLYCYTHEMIA VERA WITH METHYLENE BLUE

BEN SLUTZKY, M.D., AND ARTHUR B. VIALPANDO, B.SC., OMAHA, NEB.

(INTRODUCED BY C. M. WILHELMJ, M.D.)

The clinical application of methylene blue in the treatment of polycythemia vera is based on long-term observation of the hemolytic effect of the drug on the blood of normal dogs. This effect may be summarized as follows: (a) Methylene blue produced anemia in all dogs treated. (b) The degree of anemia was proportional to the dose of the drug administered. (c) Cessation of administration of the drug resulted in prompt return of the red blood count to normal levels. (d) Hemoglobin levels were found to parallel closely the red blood count. (e) Leukocytes remained within the normal range. (f) Bone marrow showed only evidence of increased activity. (g) There was no evidence of damage to the liver or spleen. (h) Dogs remained healthy and active at all times during the period of observation.

Noting the lack of deleterious effect of methylene blue on the general health of animals, an attempt was made to apply the hemolytic action of methylene blue on patients with polycythemia vera. Four such patients have been and are now under observation and treatment (17, 8, 7, and 5 months, respectively). One patient received no treatment previously, two patients were treated with radioactive phosphorus, and one with nitrogen mustard some months before coming under our observation. After administration of methylene blue satisfactory blood levels were reached and maintained in three patients. In one patient with biliary tract and cardiovascular disease, normal blood level was not attained because drug therapy was cautiously administered. All patients have shown considerable symptomatic improvement.

The results of the clinical observation of the effect of methylene blue may be summarized as follows: (a) Methylene blue administered orally appears to be an effective hemolytic agent in reducing the red blood count and hemo-

globin to normal levels in patients with polycythemia vera. (b) Side reactions are minimal on the doses prescribed. (c) No tolerance or sensitivity to methylene blue has been noted to date.

169. EXPERIMENTAL AND CLINICAL STUDIES ON ATHEROSCLEROSIS: DIABETES AND INSULIN IN CHICKS; ESTROGENS IN MEN WITH PREVIOUS MYOCARDIAL INFARCTION

JEREMIAH STAMLER, M.D., RUTH PICK, M.D. (BY INVITATION), AND LOUIS N. KATZ, M.D., CHICAGO, ILL.

The intensified atherogenesis supervening in diabetic patients suggested analyzing the influences of diabetes and insulin on atherosclerosis in cholesterol-fed cockerels. Forty birds were fed an atherogenic diet for 5 weeks. Ten were then sacrificed to ascertain incidence and intensity of aorta and coronary atherosclerosis. The rest were divided into 3 groups (control, insulin-treated, and hydrocortisone-treated), all on plain mash (devoid of lipid supplement). After 2 weeks, all groups exhibited resolution of hypercholesterolemia, only moderately retarded in the hydrocortisone-treated group. All groups exhibited minimal regression of aorta atherosclerosis. The controls manifested significant regression of cholesterol-induced coronary lesions. In contrast, regression of coronary lesions failed to occur in either hydrocortisone-treated, steroid-diabetic, or insulin-treated cockerels. The latter actually exhibited more extensive coronary lesions, suggesting that insulin may intensify coronary atherogenesis.

Our finding that estrogens effectively inhibit and reverse cholesterol-induced coronary atherosclerosis in chicks led to a study of the possible efficacy of estrogens in the treatment of human coronary atherosclerosis. Critical therapeutic criteria in this "double blind" study are recurrence of myocardial infarction and duration of survival of males under 50 years old who have had a single recent myocardial infarction. Higher dosages of estrogens (4.0 to 10.0 mg. per day of Premarin) tended to reduce plasma $\frac{\text{total cholesterol}}{\text{lipid phosphorus}}$ and $\frac{\beta \text{ lipoprotein}}{\alpha \text{ lipoprotein}}$ ratios. No toxic reactions occurred; side effects included gynecomastia, impotence, and loss of libido. As of Sept. 1, 1954, in the placebo group of 28 patients a second proved myocardial infarction had occurred in 6, with 3 fatalities. Of 41 patients on estrogen treatment for 2 months or longer, one had a nonfatal recurrent myocardial infarction. Neither the size of the series nor the duration of observation justifies definitive conclusions concerning the significance of these findings. However, based on these interim results, the investigation is being continued and expanded.

170. SURGICAL AND PATHOLOGIC FINDINGS IN "IDIOPATHIC PLEURAL EFFUSION"

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Pleural effusion for which no specific cause can be found is one of the most troublesome conditions encountered by the physician. Recent reports have shown that about 30 to 50 per cent of such patients develop active pulmonary tuberculosis within five years.

Twenty-four patients with a diagnosis of idiopathic pleural effusion were explored surgically at Fitzsimons Army Hospital. Fifteen of these showed unequivocal evidence of tuberculosis. In 12 of the 15 a subpleural caseous focus was found intimately bound to a thick fibrous pleural peel. It was felt that the effusion and granulomatous pleuritis were the result of an actual rupture of a peripheral caseous focus in the pleural space. In 7 of these 15 there were multiple nodules of active tuberculosis found throughout the lung (moderately to far advanced disease) which had been obscured on roentgenograms by overlying pleural thickening. It was concluded that the reason for the high rate of occurrence of pulmonary tuberculosis in these patients is that the disease begins as unrecognized pulmonary tuberculosis varying in extent from minimal to far advanced. The short period of therapy usually prescribed for pleural effusion would readily be recognized as inadequate for such parenchymal tuberculosis.

The remaining 9 patients had also been treated for tuberculous effusion, but at surgery no evidence of tuberculosis could be found. Of these, two were found to have very thick fibrous peels, probably as a result of gross hemothorax (one traumatic and one spontaneous). The remaining 7 all showed a free pleural space, a thin pleural peel trapping the lower half of the lung. In these the fluid formation was considered to be a hydrodynamic phenomenon. The cause for the fibrous pleuritis was thought to be a previous pneumothorax from the rupture of a subpleural bleb in 3, pneumonia in a bronchiectatic lobe in one, and a subpleural focus of active histoplasmosis in one. In 2 patients no cause for the pleuritis could be assigned.

The operative and pathologic findings readily distinguished those who needed therapy for tuberculosis from those in whom a simple correction of a mechanical problem was sufficient.

171. INFLUENCE OF NICOTINE, CHOLINERGIC BLOCKING SUBSTANCES, ANTISPASMODIC-SEDATIVE, AND ANTISPASMODIC-SEDATIVE-ANTACID MIXTURES ON GASTRIC (ANTRAL) MOTILITY

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Pain of peptic ulcer patients is believed by some to be due to the acid factor and by others to increased motility of the stomach. Others believe that increased acidity leads to increased motility. The latter would suggest that neutralization of the gastric acidity should decrease motility.

The effect of several substances on gastric (antral) motility was studied in a small group of peptic ulcer patients and the results are reported herewith.

The smoking of an ordinary cigarette produced in 3 of 10 patients a decrease in gastric motility, although the gastric acidity was markedly increased. Smoking of a filtered cigarette similarly decreased gastric motility, although gastric acidity was also increased, but not as markedly as after the ordinary cigarette. In the other patients the motility was unchanged except for an increase in one case.

The ingestion of 10 mg. of a cholinergic blocking agent caused a decrease of gastric motility in 70 per cent of the cases. The gastric acidity was similarly decreased in all of the patients. Following an antispasmodic-sedative mixture there was a decrease in gastric motility in 80 per cent of the patients; the gastric acidity was decreased in all.

Following the ingestion of 2 tablets of an antispasmodic-sedative-antacid mixture there was a decrease in gastric motility and gastric acidity in all patients tested.

These observations on a small group of patients seem to point to the coexistence of lowered acidity and decreased motility, except following nicotine, when decreased motility is found in some patients despite increased acidity. This finding would suggest that the pain experienced by some patients following smoking may be primarily due to increased acidity.

172. THE EFFECT OF 3,5,3′-L-TRIIODOTHYRONINE AND THYROXINE ON RENAL FUNCTION

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To evaluate the comparative effect of triiodothyronine and thyroxine on renal functions, 16 studies have been made after triiodothyronine and 4 studies after thyroxine administered orally.

Renal function was studied differentially, glomerular filtration rate (GFR) by inulin and creatinine clearances, renal plasma flow (RPF) by para-amino hippurate (PAH) clearance, and tubular excretory maximum (TmPAH). Control studies were obtained initially and repeat studies after drug administration were expressed as a per cent of the control study on each individual patient. Basal metabolic rates (BMR) were done concurrently.

After administration of triiodothyronine in doses varying from 300 to 1,200 μg there was no significant change; GFR (97 per cent of control), RPF (107 per cent of control), TmPAH (103 per cent of control), and BMR (−2 to 0). After thyroxine (6 to 24 mg.) there was an increase in GFR (110 per cent of control), RPF (134 per cent of control), TmPAH (116 per cent of control), and BMR (−10 to plus 8). The GFR:TmPAH ratio (103 per cent of control) indicates no selective enzymatic acceleration but rather an over-all metabolic increase. Two studies were done after 300 μg of triiodothyronine, two after 600 μg, seven after 800 μg, one after 900 μg, and three after 1,200 μg. There was no dosage-response relationship.

It is of interest that one-half of the triiodothyronine studies were made one hour after completion of the dose and these showed an increase in RPF to 112 per cent of control while TmPAH and GFR showed no significant change. The other one-half of the triiodothyronine studies were done more than 24 hours (and less than 48 hours) after the last dose and in these cases the TmPAH was increased to 107 per cent of the control value, while GFR and RPF showed no significant change.

In view of the absence of a similar effect to that found with thyroxine, the identity of the triiodothyronine compound is being re-examined.

173. ACTION OF ADRENAL STEROIDS ON INTESTINAL MOTILITY

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(INTRODUCED BY H. MARVIN POLLARD, M.D.)

Addison's disease is known to be associated with frequent disturbances in gastrointestinal function. This fact and, in contrast, the possibility that adrenocortical hyperactivity may be responsible for the potassium depletion which leads to paralytic ileus (Streeten, D.H.P., and Ward-McQuaid, S.N.,

Brit. M. J. 2: 587, 1952) suggested a study of the influence of adrenocortical function on bowel motility. In vitro, adrenal cortex extract has been found in small doses to have a powerful stimulant action on the propulsive motility of the small bowel, and in large doses to paralyze the intestine. That the stimulant effect may have physiologic significance was suggested by the observation in intact rats that adrenal cortex extract significantly increased motility, as reflected by the percentage of small intestine traversed by dyes administered 30 to 60 minutes before the animals were killed. This effect was reproduced in groups of eight adrenalectomized rats with DCA and corticosterone (compound B). Bowel motility was unaffected by cortisone and hydrocortisone in amounts comparable with those estimated to be present in the extract, and by adrenalectomy without hormone supplements. Larger doses of cortisone caused significant inhibition of intestinal propulsion, associated with a reduced food intake (which alone did not affect motility), a negative potassium balance, and consistent reduction in the potassium concentrations in the excised small intestine, as compared with the findings in sham-adrenalectomized controls. Administration of supplementary potassium protected the animals from the inhibitory effects of cortisone on motility. The finding that adrenal cortex extract stimulates intestinal motility in doses within physiologic limits suggests that the activity of the adrenals plays an imporant part in controlling intestinal peristalsis. The stimulant properties appear to depend on the predominantly salt-retaining steroids, probably corticosterone and possibly aldosterone (electrocortin). The inhibitory effects depend on the induction of potassium depletion which can be reproduced with large doses of cortisone in rats.

174. HISTOCHEMICAL STUDIES IN CHRONIC ULCERATIVE COLITIS

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This paper describes distinct histologic changes in the connective tissue of patients with chronic ulcerative colitis. Parallel studies were made in the normal subject. Biopsy material was obtained for study through the proctoscope from fifteen patients with ulcerative lesions of the colon and from ten normal individuals. This material was frozen freshly, dried, and mounted according to method of Gersh and Catchpole. Definite histologic changes were noted in the component structure of the epithelial cells of the mucosa of the colon in ulcerative colitis.

Clinical studies were made in the same group of patients. It is hoped that these studies may throw some light on the etiology of ulcerative lesions of the colon.

175. POLYDIPSIA AS A MECHANISM FOR THE PRODUCTION OF CORTISONE "DIURESIS"

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Studies were made on a patient with anterior and posterior pituitary hypofunction. On a constant 1.9 Gm. sodium diet, 100 mg. cortisone a day was given for 5 days. Intake and output almost doubled in 12 hours and tripled in 4 days. Serum sodium was markedly increased in 12 hours, then returned to control values.

Twelve hours of fluid deprivation produced no distress; urine flow dropped from 6.5 ml. per minute to 1.0 ml. per minute; concentration rose to 422 m/osm/L. (freezing point determinations). Corresponding figures pretreatment were 2.8 to 1.0 ml. with a rise to 464 m/osm/L. Polydipsia, not polyuria, appeared primary, as in prolonged DOCA or cortisone administration in many experimental animals.

The rapid onset and initial polyuria exceeding intake suggested renal factors. Diuresis could not be correlated with daily endogenous creatinine clearance or $GFR_{(in)}$ and $GFR_{(CR)}$ by standard techniques, although renal plasma and blood flow increased markedly. Intravenous hydrocortone caused a slight increase in $GFR_{(in)}$ but a very large increase in urine flow. Urinary ADH assays showed no decrease in the small amount of ADH-like material present. There was increased sensitivity to pitressin. Total solute excretion rose slightly, but not in proportion to the rise in urine output. The increase occurring was attributed to increased urea output; urine sodium decreased.

It is concluded that decreased tubular water reabsorption and increased salt retention stimulated thirst with resulting water diuresis. This depended on an adequate thirst mechanism and probably on the particular type of brain damage.

176. INTRACELLULAR AND EXTRACELLULAR FLUID AND ELECTROLYTE ALTERATIONS IN CIRRHOSIS OF THE LIVER WITH EDEMA AND ASCITES

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The present study was undertaken to clarify the pathogenesis of the ascites and edema in cirrhosis of the liver by investigating the distribution of intracellular and extracellular electrolytes and water in four patients, correlating the chemical findings in skeletal muscle biopsies with the results of metabolic balance studies; and determining the pattern of loss of electrolytes and water induced by a low-salt diet, an exchange resin and by a mercurial diuretic. Similar observations were made for control purposes in a healthy young man.

The gastrocnemius and deltoid muscles of the edematous patients contained an increased amount of extracellular water; intracellular water was not increased significantly; the fluid content of the gastrocnemius exceeded that of the deltoid, reflecting the influence of gravity. After therapy had decreased the edema and ascites, the extracellular fluid diminished in the gastrocnemius, but not in the deltoid muscle; both remained higher than normal. The intracellular water content did not change significantly after therapy. The pattern of loss of the edema was consistent with the movement of isotonic, extracellular fluid.

Complete restitution of electrolyte and fluid balance to normal was not observed in the present study; hence additional trends may become evident as this objective is accomplished. Nevertheless, the data generally indicate that the edema and ascites in cirrhosis of the liver are isotonic expansions of extracellular fluid; in contrast to the edema of congestive heart failure, characterized as an hypotonic expansion of both intra- and extracellular phases. The pathogenesis of the edema and ascites in cirrhosis of the liver appears to depend upon a mechanism capable of inducing an isotonic expansion of the extracellular phase of body water.

177. THE CREATINE CONTENT OF BIOPSIED HUMAN HEART MUSCLE

DON C. SUTTON, M.D., GEORGE C. SUTTON, M.D. (BY INVITATION), RAYMOND RONDINELLI, B.S. (BY INVITATION), AND EGBERT H. FELL, M.D. (BY INVITATION), CHICAGO, ILL.

Previous reports have established the normal levels of creatine in whole blood and skeletal muscle and also the changes occurring during heart failure using Folin and Wu's picric acid method as modified for the photelometer by Hoffman. Normal blood values agreed with those found by Beard—approximately 3 mg. per cent of whole blood. We established normal skeletal muscle creatine levels of approximately 350 mg. per cent.

Cardiac surgery offers the possibility of obtaining biopsies from the heart. The creatine level in sections of seventeen auricular appendages averaged 456.8 mg. per cent. The creatine values in four biopsies of the left ventricle averaged 418 mg. per cent. These values are much higher than those found by Meyers in autopsy material. We believe this is the first report of the level of creatine in nearly normal heart muscle.

178. CREATINE VALUES OF HEART MUSCLE BIOPSIES IN RABBITS

DON C. SUTTON, M.D., GEORGE C. SUTTON, M.D. (BY INVITATION), JOHN CHENG, M.D. (BY INVITATION), AND RAYMOND RONDINELLI, B.S. (BY INVITATION), CHICAGO, ILL.

Meyers reported the average creatine content of the left ventricle as 205 mg. per cent in autopsied material. One patient, from whom an auricular appendage biopsy had been taken, died on the table. In this case the auricular appendage contained 570 mg. per cent. The patient expired 15 minutes later. Immediate biopsy of the ventricular muscle contained 290 mg. per cent of creatine.

Under ether anesthesia, the whole blood and biopsies of the auricle and left ventricle were taken from large rabbits. The animals were kept alive 10 to 15 minutes and the same procedures carried out immediately after death. In five animals before death, the average level of whole blood creatine was 2.42 mg. per cent and the average level of creatine in the auricles 406 mg. per cent and the left ventricle 386.6 mg. per cent.

Immediately after death the average of creatine in the auricle was 357.4 mg. per cent, and in the left ventricle 279.8 mg. per cent. Three animals were fed from 200 to 500 mg. of alpha-tocopherol during the week before study. The average creatine content of the whole blood before death was 3.7 mg. per cent, of the auricles 450.4 mg. per cent, and the left ventricles 449.3 mg. per cent. After death the average creatine values in the auricle were 436 mg. per cent, and the left ventricle 418.6 mg. per cent. In two animals without alpha-tocopherol, one hour after death, the auricle values did not fall materially while the ventricular creatine decreased 100 mg. per cent below those immediately after death. There was no change in the values of creatine in either auricle or left ventricle in the alpha-tocopherol treated animals one hour after death. Addition of 500 mg. of alpha-tocopherol to 100 ml. of blood introduces no error in the determination of total creatine.

These studies further emphasize the possibility that the creatine levels in whole blood are of prognostic value as suggested by earlier studies in patients with cardiac failure.

179. ELECTROCARDIOGRAPHIC OBSERVATIONS DURING QUINIDINE ADMINISTRATION TO PATIENTS WITH ATRIAL FIBRILLATION

II. Atrial Flutter as an Intermediary Stage Before Conversion to Normal Sinus Rhythm

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Quinidine sulfate was given orally 125 times to 92 patients with chronic atrial fibrillation in an attempt to convert the abnormal rhythm to sinus rhythm. Eighty attempts were successful, while 45 attempts failed. This represents a conversion rate of 64 per cent.

Among the 80 instances of conversion 23 (28.8 per cent) progressed through a stage of atrial flutter. The study should be divided into two periods: the earlier period when electrocardiographs were taken at rather infrequent intervals and the later period when daily electrocardiographs were obtained. Eighteen successful conversions were obtained during the earlier period; only two instances in both of which daily electrocardiographs were recorded demonstrated a transient stage of atrial flutter, as atrial fibrillation was converted under the influence of quinidine to normal sinus rhythm. In the later part of our study, which contained 62 successful conversions, daily electrocardiographs were recorded and 21 progressed through a stage of atrial flutter before sinus rhythm was restored. Eighteen relapses occurred while the patient was still under observation, and 13 again progressed through a stage of atrial flutter with increasing atrial rates as the levels of plasma quinidine declines until the rhythm was finally atrial fibrillation.

Among the 45 cases of failures, 24 (53.3 per cent) were changed into atrial flutter with the atrial rates generally fluctuating in inverse ratio to the plasma levels of quinidine. All except one went back to atrial fibrillation as soon as as the plasma levels of quinidine showed appreciable decline.

The frequency with which atrial flutter is noted as cases of atrial fibrillation are treated with quinidine for conversion to sinus rhythm probably depends on the diligence on the part of the investigator and the frequency with which electrocardiograms are taken as the quinidine level in blood rises and the conversion time approaches. Our observation would tend to support the unitary nature of atrial fibrillation and flutter in which the only difference is the rate of discharge of the ectopic focus. Quinidine decreases the rate of discharge from the ectopic focus.

180. STUDIES OF THE METABOLISM OF WATER AND ELECTROLYTES IN PATIENTS WITH CONGESTIVE HEART FAILURE AND WITH CIRRHOSIS OF THE LIVER

Peter J. Talso, M.D., George W. Ferenzi, M.D. (By Invitation), Irvin H. Strub, M.D. (By Invitation), and Norma Spafford, B.S. (By Invitation), Chicago, Ill.

The mechanisms of salt and water retention in heart failure and in patients with Laennec's cirrhosis are as yet incompletely understood. Therefore, a study of total body water, exchangeable sodium, sodium space, thiocyanate space, and serum osmotic activity in edematous patients was undertaken.

Exchangeable sodium was determined with Na^{22}, total body water by dilution of N-acetyl-4-amino antipyrine, and thiocyanate space by dilution. The sodium space was calculated allowing 4½ and 24 hours for equilibrium. Osmotic activity was determined by measuring the freezing point depression of serum.

Studies were performed on 14 normal controls, 14 patients with congestive heart failure of varying degrees of severity and in 5 patients with Laennec's cirrhosis.

The results revealed that sodium is retained in excess of water both in congestive failure and in cirrhosis of the liver, but that in congestive failure this discrepancy is more marked than in cirrhosis of the liver. Despite the retention of sodium in excess of water several instances of hyponatremia were observed both in patients with heart failure and in those with cirrhosis. Those patients who exhibited hyponatremia also had a decreased serum osmotic activity.

The thiocyanate space was consistently larger than the Na^{22} space by approximately 43 per cent both in the patients and in the control subjects; however, both the thiocyanate space and the sodium space were larger in the edematous subjects than in the controls. When the total body water, thiocyanate space, and sodium space were compared in the edematous cases and in the controls, it was noted that the increase in sodium space and thiocyanate space was proportionately greater than the increase in total body water.

181. THE ROLE OF CARBONIC ANHYDRASE IN THE PRODUCTION OF HYDROCHLORIC ACID

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Carbonic anhydrase, which is found in high concentration in the gastric parietal cells, has been postulated to play a role in hydrochloric acid secretion by making $[H^+]$ rapidly available through catalysis of carbon dioxide hydration and its ionic breakdown. If carbonic anhydrase is actively involved in the process of HCl secretion, the use of carbonic anhydrase inhibitors should have an inhibitory effect upon the formation of HCl. No effect on HCl secretion was observed, however, when the inhibitors sulfanilamide and thiophine-2-sulfonamide were administered.

Acetazoleamide (Diamox), a heterocyclic sulfonamide, is 440 times more potent than sulfanilamide as an inhibitor of carbonic anhydrase. Histamine-stimulated HCl secretion in Heidenhain dogs was inhibited by acetazoleamide (20 mg. per kilogram I. V.). Previous studies of its inhibitory effect on HCl secretion in human beings were inconclusive.

The antisecretory effects of acetazoleamide were evaluated using the one-hour basal gastric secretion. Studies were done before and after acetazoleamide administration, 250 mg. q.i.d. orally (14 mg. per kilogram). Fifty tests were carried out on 24 peptic ulcer patients with the following results. Secretory volume: control mean 57.2 ml.; acetazoleamide mean 55.2 ml.; mean difference 2.0 ml. ($t = 0.3676$, $0.8 > p > 0.7$). HCl recovery: control mean 75.4 mg. per hour; acetazoleamide mean 29.5 mg. per hour; mean difference 45.9 mg. per hour ($t = 4.596$, $p < 0.001$).

Acetazoleamide, a potent carbonic anhydrase inhibitor, had no effect upon secretory volume but exerted significant inhibitory effect on HCl recovery. It appears that carbonic anhydrase plays the following role in the secretion of HCl. The enzyme is concentrated largely in the parietal cells where its action is to catalyze the reaction $H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$. Carbonic anhydrase thereby participates in the reverse chloride shift in which

$[Cl^-]$ is transferred from the blood to the parietal cell and $[HCO_3^-]$ is returned to the gastric venous blood. The $[H^+]$ remaining in the parietal cell could participate in the formation of HCl. Acetazoleamide appears to inhibit HCl formation by decreasing this source of $[H^+]$. The relation of these observations to the current concepts of the physiology of gastric secretion will be discussed.

182. STUDIES OF EXCRETION OF RADIORUBIDIUM (Rb^{86}) AND POTASSIUM IN MAN WITH AND WITHOUT CONGESTIVE HEART FAILURE

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Lack of a long-life isotope of potassium has handicapped tracer studies of potassium, therefore Rb^{86} ($T_{½}$ 19.5 days) was evaluated as a tracer of potassium. Studies included observations of rates of turnover for erythrocytes, plasma, urine, stools of Rb^{86}, K^{42}, K^{39}, Na^{23}, Cl^{35}, and water for man with and without congestive heart failure under controlled metabolic conditions.

For the first two hours following simultaneous administration of K^{42} and Rb^{86} to two subjects it was found: (a) Appearance time of both in urine was similar to those of other isotopes studied. (b) Excretion (urinary concentration, clearance, excretion as a percentage of that present) of Rb^{86} was only 52 and 68 per cent that of K^{42} but was qualitatively similar. (c) Rate of transfer into urine was much slower for Rb^{86} than for K^{42} although they disappeared from the plasma at essentially the same rates. (d) Both manifested the phenomenon of "skimming," i.e., excretion without equilibration with the larger compartments.

Excretion of Rb^{86} and K^{39} for 36 to 49 days after administration of Rb^{86} to four subjects (two controls and two with congestive failure) showed: (a) Daily rates of excretion of Rb^{86} and potassium ranged from 0.5 to 2.0 per cent of that calculated to be present in the body. Potassium excretion exceeded Rb^{86} excretion. Variations were concordant but the quantitative relationships were not constant. (b) The urinary excretion of all substances measured varied considerably. (c) From 15.2 to 44.3 per cent of the injected doses was recovered when urinary counts reached background. (d) Twenty to 25 per cent of the tracer excreted was recovered in normal stools; approximately 50 per cent in diarrheal stools. (e) Increased intake and output of potassium did not increase excretion of Rb^{86} proportionately. (f) Mercurial diuretics increased excretion of Rb^{86} and potassium less than that of sodium and chloride. Although Rb^{86} was not a precise tracer of potassium, because of its long life it can and did provide significant information about rubidiokinetic and kaliokinetic phenomena not obtainable with K^{42}.

183. THE SPECIFIC DIAGNOSIS OF POLIOMYELITIS BY USE OF A HUMAN EPITHELIAL CELL, STRAIN HeLa (GEY), IN CONTINUAL CULTURE

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The 1953 poliomyelitis epidemic in Minnesota provided opportunities to: (a) evaluate the usefulness of continually cultured human epithelial cancer cells, strain HeLa (Gey), for routine virus isolation and (b) assess the place of a serologic test in the laboratory diagnosis of poliomyelitis. Two or more

fecal samples from each of 513 cases of paralytic and "non-paralytic" poliomyelitis were tested for virus by transfer to two HeLa tube cultures. This minimal effort resulted in isolation of poliomyelitis virus from 78 per cent of paralytic cases and from 42 per cent categorized as "non-paralytic." Recovery of virus ranged from 90 per cent for patients more than 10 years old with bulbar spinal disease, to 27 per cent for patients diagnosed clinically as "non-paralytic" poliomyelitis. These findings suggest that another causative agent is responsible for many instances of the latter condition. It is noteworthy that: (a) samples of feces taken within three days of the termination of fever were most likely to yield virus; (b) about half of the patients excreted virus for from 2 to 3 weeks while 18 per cent excreted for 5 or more weeks; (c) the monthly percental positive isolations were constant.

The presence of type specific antibody in patients' serum was established with a neutralization test conducted with fourfold serum dilutions mixed with 25 to 100 $TCID_{50}$ virus units. For many patients the antibody titer did not rise significantly during hospitalization. In other cases, a homotypic rise was accompanied by heterotypic increase. Serologic findings helped eliminate poliomyelitis virus as the causative agent in many non-paralytic cases. The implications of the 1953 findings will be discussed. Present studies have resulted in technical modifications which should increase the incidence of virus recovery.

184. A STUDY OF PULMONARY ARTERY PRESSURE IN ACUTE "NEUROGENIC HYPERTENSION" IN DOGS

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With the Technical Assistance of A. Bennett, B.A. (By Invitation), and M. Finley, B.S. (By Invitation)

Concomitant changes of the pulmonary artery pressure were studied in dogs rendered hypertensive by section of the pressor regulator nerves (under pentobarbital anesthesia). A mercury manometer was used for recording of the mean systemic arterial pressure and a Sanborn electromanometer for the pulmonary artery pressures.

The mean systemic arterial pressure and the cardiac rate showed a graded increase with the following procedures in this order: Group I occlusion of common carotid arteries for one minute, Group II occlusion of common carotid arteries for five minutes, Group III denervation of carotid sinuses, and Group IV section of all the four carotid and aortic regulator nerves. The pulmonary artery pressures remained unchanged in all the 5 dogs of Group I, 2 of the 5 dogs of Group II, and 3 of the 9 dogs of Group III. The rest of the dogs of Groups II and III and all the 5 dogs of Group IV showed significant elevation in the pulmonary systolic, diastolic, and mean pulmonary artery pressures and the mean pulmonary "capillary" pressure, but no increase in the systemic venous pressure.

The data demonstrate that pulmonary hypertension occurs regularly with acute "neurogenic hypertension." The amount of increase in the pulmonary artery pressure, like that in the systemic artery pressure, is dependent upon the extent to which the pressor nerves are sectioned. This would indicate that a simple quantitative effect is exerted through the graded elimination of the depressor mechanism. Although the response in the pulmonary circulatory system is less sensitive than that of the systemic, the data also suggests that both the systemic and pulmonary artery pressures are influenced by the

pressor regulator nerves through (a) the reflex effect of these nerves on the sympathetic tone or (b) change in the sympathetic tone plus a humoral effect such as one due to the liberation of pressors from the adrenal medulla. Additional work is being done. The finding of pulmonary hypertension in this experiment again emphasized the difference between acute "neurogenic hypertension" and "renal hypertension" of Goldblatt dogs, in which the pulmonary artery pressures remain normal even during the earliest stage of systemic hypertension (Kenoyer and Gregory, 1953).

185. PRELIMINARY REPORT OF THE EFFECT OF CORTICOTROPIN (ACTH) AND CORTISONE ON THE ELECTROPHORETIC PATTERNS OF PLASMA OR SERUM PROTEINS OF CHILDREN WITH RHEUMATIC FEVER

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A great deal of evidence points to Group A hemolytic streptococci as being related to the cause of rheumatic fever. This does not explain, however, why only certain children develop the disease after having had streptococcal infections. It is well known that there is a high familial incidence present and that heredity may play a role. In addition, evidence is accumulating to show that the highest incidence of the disease with more serious complications occurs in children whose general health is suboptimal prior to the onset of the disease. Our research, therefore, has been directed toward the host factor, and inasmuch as we have been primarily interested in nutritional and metabolic studies, the problem has been approached from this standpoint. It was thought that observations of the changes in the protein fractions of the blood during hormonal therapy would increase our knowledge of the actions of these drugs and provide added insight into the pathologic physiology of the disease.

This study is based on the electrophoretic analyses of the protein of 24 plasma or serum samples of 9 children with definite acute rheumatic fever. All of these patients were treated with corticotropin or cortisone.

Blood for electrophoretic analyses was obtained prior to therapy from all patients in the early or late acute stage of the disease. Additional samples were taken at later stages in the disease. Each protein fraction of the electrophoretic pattern is reported on a percentage basis.

Although further work must be done, our data indicate that hormonal therapy exerts a definite effect on albumin, alpha$_1$ globulin, beta globulin, and gamma globulin. The results show that the rapid return of albumin to normal is a compensatory response resulting from the decrease in the percentage of globulins.

Alpha$_1$ globulin returns to normal more rapidly than in the nonspecifically treated group. Beta globulin either remains elevated or becomes even more elevated during the course of treatment. Gamma globulin decreases rapidly, sometimes to subnormal levels. Hormonal therapy has little effect on the alpha$_2$ globulin or mucoprotein tyrosine.

186. URINE ADSORBATE WITH FIBRINOLYTIC AND THROMBOPLASTIC PROPERTIES

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(Introduced by Matthew Block, M.D.)

Fibrinolytic and thromboplastic properties of human urine have each been reported. No stable preparation with either, or both, activities has been described. Such a preparation may be obtained if pooled urine of normal males, acidified to pH 4.5, is adsorbed on $BaSO_4$ (5 Gm. per 1,000 ml. urine). The washed $BaSO_4$ is eluted several times by 3.8 per cent tribasic sodium citrate (5 ml. per 1,000 ml. urine each time), the dialyzed eluates saturated to 60 per cent with $(NH_4)_2SO_4$ and the precipitate collected and dried. The resulting powder, readily soluble in buffered saline, has fibrinolytic and thromboplastic properties, which it retains for months when stored in a desiccator. It will (a) activate human plasminogen to plasmin in 15 to 45 minutes; the activation is less complete than with streptokinase; (b) produce fibrinolytic action by less than 0.000015 mg. on unheated fibrin plates, but 0.150 mg. does not have fibrinolytic action on heated fibrin plates. This demonstrates that the substance is a fibrinolysokinase (an activator of plasminogen). Furthermore, the fibrinolytic activity against human plasma clots is considerably greater than that of commercial crystallized trypsin.

Normalization of the clotting time and prothrombin consumption of hemophiliac plasma is demonstrable by as little as 20 μg per milliliter. Continuous recording of the active clotting process (thrombelastography) shows that beside the onset of fibrin formation, the speed of this formation and its intensity are also influenced. The activity of as little as 5 μg per milliliter can be demonstrated by thrombelastography.

If the amount of urine adsorbate added to hemophiliac plasma is increased than by thrombelastography, a normal clotting pattern is attained, followed by a quick dissolution of the clot. This fibrinolysis depends on the concentration of the urine adsorbate. These details of the action of the thromboplastic and fibrinolytic components are demonstrable only by thrombelastography. The significance of these findings is discussed.

187. LONG-CONTINUED INTRAVENOUS ADMINISTRATION OF HISTAMINE IN CASES OF LESIONS OF THE OPTIC NERVE, RETINA, AND CHOROID

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The long-continued administration of histamine has been employed in a rather large group of cases in the past eleven years. This group consisted of cases of residual optic or retrobulbar optic neuritis and cases of scarred chorioretinal lesions involving the macula which had been thought by competent observers to be quiescent or healed, and not amenable to further treatment.

We employed a 1:250,000 solution of histamine base which was administered intravenously by the drip method at rates varying from 18 to 60 drops per minute. Treatment for one and a half hours was carried out daily, or at less frequent intervals, for a few months to over nine years. No untoward reaction has been observed. The maximal number of treatments administered to a patient with retrobulbar neuritis was 978; 895 is the maximum number of treatments administered to a patient with central chorioretinitis.

Lesions of the Optic Nerve.—Good reading vision was obtained in 61 per cent of all the eyes treated and in 12 of 17 eyes (71 per cent) with acute retrobulbar neuritis. This percentage would have been higher had all cases been included in which vision returned to normal before the patient had received 60 intravenous injections of histamine.

Chorioretinitis.—Histamine is particularly effective in the treatment of primarily edematous lesions such as central angiospastic retinopathy.

In cases of hemorrhagic and exudative chorioretinitis with macular involvement, treatment with histamine usually does not result in improved vision until the lesion is ophthalmoscopically quiescent.

Maintained improvement in vision was secured in 11 of 16 eyes (69 per cent) and good or useful reading vision was restored to 8 (50 per cent). Scotomas remained in the central or paracentral fields in all cases.

Chorioretinal Degenerations.—Definite maintained improvement was noted in only 4 of the 22 eyes treated. Good reading vision was not restored to any eyes but was maintained in 6 through the presence of a small preserved isle of central vision.

The exact manner in which histamine acts in these "healed" lesions of the optic nerve, choroid, and retina is not clearly understood, but the results obtained have demonstrated that the loss of vision in such cases is not always as permanent as has been previously supposed.

188. THE EFFECT OF PHOSPHATIDE ON THE DISAPPEARANCE OF FREE CHOLESTEROL FROM INCUBATED SERA, NORMAL AND HEPARINIZED

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(INTRODUCED BY HENRY R. JACOBS, M.D.)

When normal human serum is incubated for twenty-four hours or longer at 37° C. the free cholesterol diminishes without change in the total cholesterol. This effect is increased if an emulsion of soy bean phosphatides is added to the serum before incubation. The same result appears to even greater degree if an emulsion of crystalline synthetic lecithin is used. The administration of a small dose of heparin by vein changes the outcome. Then the spontaneous disappearance of free cholesterol (without added phosphatide) tends to be inhibited. The effect of added phosphatide is also diminished or reversed. These antagonistic actions of heparin appear within five minutes and persist for about three hours. Thirty-one healthy young subjects were studied.

189. THE COMBINATION OF ERYTHROMYCIN AND CHLORTETRACYCLINE FOR THE TREATMENT OF BACTERIAL ENDOCARDITIS

BURTON ARMIN WAISBREN, M.S., M.D., MILWAUKEE, WIS.

The purpose of this report is to point out that the combination of erythromycin and chlortetracycline may be effective in the treatment of cases of endocarditis that do not respond to more conventional therapy.

We first found this combination of antibiotics to be effective in vitro against a strain of enterococcus isolated from a patient who was not responding to therapy. Further study revealed that when the patient was given 1 Gm. of erythromycin by vein her serum inhibited the enterococci in a 1:2

dilution. The same dilution of her serum was inhibitory after she was given 0.5 Gm. of chlortetracycline by mouth. When she was given both drugs simultaneously the strain of enterococcus was inhibited by a 1:16 dilution of her serum. Recovery followed the daily administration of 2 Gm. of erythromycin by vein and 2 Gm. of chlortetracycline by mouth for six weeks.

In vitro studies with five additional strains of pathogenic enterococci revealed further instances of potentiation with erythromycin and chlortetracycline and that this combination was often superior to that of penicillin and streptomycin.

Three other cases of bacterial endocarditis then were treated successfully with erythromycin and chlortetracycline after they had not responded to the combination of penicillin and streptomycin.

These four cases and the in vitro studies will be presented.

190. EXPERIMENTAL HYPERTENSION PRODUCED BY CONSTRICTION OF THE CAROTID SINUS AREA

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Bilateral constriction of the carotid sinus area by a plastic clamp with a two-thirds reduction of the palpable pulse of the sinus and the internal and external carotid arteries produced a significant, sustained increase in arterial blood pressure in 10 out of 11 dogs. Six of the 10 dogs showed a gradual increase over a period of 2 to 4 months. The other 4 dogs showed a rapid increase during a period of 1 to 3 weeks. Three showed increased irritability and aggressiveness for 3 to 4 months following operation, but the pressure increase persisted after the nervous tension disappeared. Otherwise there were no gross clinical changes. Heart rates have remained within normal limits. Urinalyses, appetites, and body weights have remained normal during the 4 to 17 months of blood pressure increase. Preliminary studies on two of the carotid sinus area hypertensive dogs demonstrated that the vagi were intact. Two control dogs subjected to bilateral implantation of the plastic clamps immediately lateral to the carotid sinus showed no significant change in blood pressure. Likewise, 10 untreated control dogs observed in the laboratory during the same 17-month period showed no significant change in their normotensions. The pathogenesis of this experimental hypertension is unknown. Possibly altered carotid sinus nerve function or a cerebrogenic mechanism may be involved.

191. CLINICAL STUDIES OF SECONDARY AMYLOIDOSIS

Maurice H. Wald, M.S., M.D., Winnetka, Ill.

(Introduced by Henry R. Jacobs, M.D.)

The results of a survey of thirty cases of amyloidosis secondary to tuberculosis are presented. There was a marked preponderance of white males in the series. Females seem to resist the complication of amyloidosis and survive it longer. The average age of the group was 36.6 years and the average duration of tuberculosis to the presumed onset of amyloidosis was 4.6 years. Albuminuria and hepatomegaly were almost universally present, and were, therefore, prime diagnostic signs.

Tests of hepatic function revealed impairment in many cases. Most significant were diminished esterification of cholesterol in 13 of 20 cases, elevation of cephalin flocculation to 2 plus or more in 11 of 12 cases, and B.S.P retention above 10 per cent in 8 of 23 cases.

Renal function was impaired in the majority of cases and three known deaths in uremia occurred. Notable was the absence of the classical syndrome of "nephrosis" save in one case.

The plasma proteins were found normal or supernormal in 24 of 28 cases tested, while the albumin-globulin ratio was depressed in 21 of these. To account for these and associated findings a concept is offered whereby they may be viewed as the net effect of (a) stimulation of globulin formation by infection, (b) albumin loss by reason of albuminuria, and (c) the depressing effect of all protein production by hepatic impairment.

The most striking finding of this study is the marked improvement in the prognosis of secondary amyloidosis since the introduction of the new methods of tuberculosis treatment. These observations were made during the period of therapeutic transition and the seven cases of long-term survival, including two cases of regression of amyloidosis, clearly show the important change in prognosis.

192. THE EFFECT OF VARIOUS DISEASE STATES ON THE INTRAVENOUS GLUCOSE TOLERANCE TEST AND THE GLUCOSE INSULIN TOLERANCE TEST

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Determinations of the rate of disappearance of glucose from the blood after intravenous administration, using the Glucose Tolerance Test (G.T.T.) and Glucose Insulin Tolerance Test (G.I.T.T.), have been made in normal individuals and patients with metabolic and endocrine disorders, both organic and iatrogenic, using adrenal steroids. The rates of disappearance are determined according to principles previously established, applying the first order reaction velocity equation to the disappearance of injected glucose from the blood.

Glucose was given intravenously, 25 Gm. over a three-minute period, and sampling done for one hour. Insulin, 0.05 unit per kilogram, was used after the glucose injection in the G.I.T.T. Rates are expressed as milligram per cent per minute.

The following summarizes most of our data: The range of normal G.T.T., 21 patients, is 1.9 to 3.3 mg. per cent per minute and G.I.T.T. is 4.6 to 7.3 mg. per cent per minute; mild diabetics, 5 patients, 1.2 to 1.6 and 3.2 to 6.6; severe diabetics, 10 patients, 0.7 to 1.1 and 1.5 to 6.9; cirrhosis, 9 patients, 0.8 to 1.8 and 2.8 to 5.3; hypopituitarism, 7 patients, 1.7 to 5.1 and 2.7 to 6.8. During long term ACTH and cortisone therapy, 15 patients, the range is 0.8 to 2.4 and 1.7 to 6.0; while for short term ACTH therapy, 7 patients, prior to administration G.T.T. is 1.9 to 3.5 and during treatment 1.2 to 2.7. P values for this data are $\leqq .01$.

The disappearance rate of glucose in normal individuals is constant and reproducible and there are statistically significant changes characteristic of various disease states. Interpretation by this method allows more accurate differentiation between normal and borderline states of glucose utilization and permits evaluation of results by a single numerical expression.

Using the G.I.T.T. and the absolute change in rate between the G.T.T. and G.I.T.T., the degree of responsiveness of the glucose utilization mechanism to

insulin is established. This degree of responsiveness does not necessarily correspond to the patient's hypoglycemic responsiveness, which involves other compensatory mechanisms.

193. CLINICAL AND METABOLIC EFFECTS OF ALDOSTERONE IN RHEUMATOID ARTHRITIS

L. Emmerson Ward, M.D., Howard F. Polley, M.D., Charles H. Slocumb, M.D. (By Invitation), Philip S. Hench, M.D., Harold L. Mason, Ph.D., and Vernon R. Mattox, Ph.D. (By Invitation), Rochester, Minn.

Aldosterone in sesame oil was administered intramuscularly every 6 hours for 6 days to one man and one woman, each of whom had active rheumatoid arthritis. Total daily doses were 200, 250, 400, 400, 800, and 280 μg for the man, 800, 800, 800, 600, 1,000, and 640 μg for the woman. No antirheumatic effect could be detected clinically with these doses in these patients. The erythrocytic sedimentation rate of the man did not change; that of the woman decreased slightly but probably not significantly.

There was retention of fluid during administration of aldosterone. This was manifested by gain in weight of 1.4 kilograms by the man and 2 kilograms by the woman, with slight edema and slight increase in plasma volume in the woman. Excretion of urinary sodium and chloride by the woman decreased during administration of aldosterone. Discontinuation of administration was followed by diuresis within 1 to 2 days; body weights returned to original levels within 2 to 3 days. Concentrations of serum potassium decreased slightly in the man (5.0 to 3.9 meq. per liter) and remained unchanged in the woman. Concentrations of sodium, chloride, carbon dioxide, calcium, and phosphate in the serum and urea in blood were not significantly altered. No significant changes occurred in the glucose tolerance, ratio of albumin and globulin in the serum, urinary excretion of 17-ketosteroids and corticosteroids, basal metabolic rate, electrocardiogram, electroencephalogram, or blood pressure. The eosinophil count of the woman did not change 4 and 24 hours after administration of aldosterone was begun from that before administration. Balance studies of metabolism of sodium, potassium, chloride, nitrogen, calcium, and phosphate, including study of effects of aldosterone on urinary excretion of these substances, will be reported.

Doses used in this preliminary study were considerably larger than those reportedly used effectively for patients who had Addison's disease. However, the range of dosage necessary for production of antirheumatic effects, if any, may not have been reached by these doses. When supplies of aldosterone permit, larger doses will be employed for further study of antirheumatic and other effects.

194. DEGENERATIVE DISEASE REVERSIBLE WITH DEPLETION OF STORAGE METABOLITES

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(Introduced by Theron G. Randolph, M.D.)

Geriatric patients maintained on the rigid Kempner rice diet undergo progressive reversal of existing, degenerative disease. These changes include the reversal of arthritic and arteriosclerotic processes, clearing of allergic manifestations, return to a normal endocrine balance, and improvement in hearing and vision.

Since medication is discontinued as soon as possible, the progressive improvement of patients on this schedule must be due to the dietary restriction and the resultant depletion of storage metabolites within the body as a whole. The predominant carbohydrate character of the rice diet suggests that the depletion is primarily of fat and protein storage forms.

Carbohydrate and fat storage has been largely explained as regards mechanism and tissue depot. Protein storage is recognized, but the storage form, enzymology, and biochemistry remain unsolved. I have felt collagen and fibrous tissue and amorphous ground substance are the principal depots of protein storage.

Acceptance of this concept for purposes of clinical study furnished a ready explanation for the symptoms arising in patients on protein depletion schedules. The usual complaints are fatigue, tachycardia, epigastric pain, neuralgias, bursitis, arthralgia, and hydroarthrosis. These adverse symptoms subside in intensity during the initial six months and are followed by increased energy production and exercise tolerance. Fasting or restriction of diet to fruit and fruit juice shortens the duration of neuralgic and arthralgic distress episodes.

The role of salt restriction in diet has not been clarified. Salt depletion apparently retards hyalinization, but the symptomatic improvement obtained with alkalinization using sodium bicarbonate raises a question regarding the role played by chlorine. It would appear that chlorine assumes characteristics usually attributed to bromine and iodine.

Clinical studies have been in progress for the past three years. Medication is largely eliminated, specific vitamin therapy is prescribed, and aspirin is usually sufficient for analgesia. Physiotherapy is utilized as an adjunct to active exercise. Serial x-rays, laboratory data, electrocardiograms, etc., are being compiled. The modifications of the rice diet which are being evaluated are similar to the dietary recommendations in force during the first three decades of this century.

195. DETERMINATION DURING SURGERY OF COMPLETENESS OF CLOSURE OF ATRIAL SEPTAL DEFECTS

WILLIAM H. WEIDMAN, M.D. (BY INVITATION), JOHN W. KIRKLIN, M.D. (BY INVITATION), E. JAY FIELDMAN, M.D. (BY INVITATION), AND EARL H. WOOD, M.D., PH.D., ROCHESTER, MINN.

Ivalon sponge was used to close atrial septal defects in 20 patients with use of the well technique of Gross. Postoperative catheterization on some of the initial patients revealed that significant arteriovenous shunts could persist via unsuspected residual defects which, apparently due to small size, had escaped detection by palpation during surgery. Hence, hemodynamic studies were performed on 13 patients both preoperatively and during closure of the defect. The following technique was devised to determine whether closure had been complete.

A polyethylene catheter was inserted through the atrial well, into the venae cavae and right ventricle; pressures were recorded and samples of blood withdrawn through a cuvette oximeter for immediate determination of the oxygen saturations before and after placement of the sponge over the defect. In addition, arterial dye-dilution curves were recorded and projected on a ground-glass screen for immediate visual interpretation.

Presence of an arteriovenous shunt was demonstrated by increased oxygenation of blood entering the right ventricle and by prolongation of the dis-

appearance slope of the dye curve. A venoarterial shunt was shown by an abnormally short appearance and abnormal initial deflection of dye.

The possibility of large shunts through small openings was demonstrated by the above technique. After closure of large defects, except for the small slit remaining before suture of the posterior edge of the sponge to the septal remnant, data on oxygen saturation of the blood demonstrated that the arteriovenous shunt may decrease only slightly (less than 10 per cent of the pulmonary flow). Likewise, arteriovenous shunts of more than 30 per cent persist via small defects, seemingly inconsequential in size, which initially were difficult for the surgeon to identify by palpation alone. After placement of additional sutures through such defects, data on oxygen saturation of the blood and the dye-dilution curves have indicated complete closures.

Five patients had small residual arteriovenous shunts. As the study progressed, the proportion of defects closed completely increased. In 8 patients proof of complete closure was obtained while they were still on the operating table. Findings at operation were confirmed in the 6 patients catheterized postoperatively.

196. COMBINED SPLENIC ASPIRATION AND SPLENOGRAPHY

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The value of splenic aspiration as a diagnostic procedure is recognized. Visualization of the portal and splenic veins combined with examination of the splenic aspirate is occasionally helpful in the diagnosis of splenomegaly of undetermined etiology. This combined procedure was employed in fifteen patients. In seven of these patients visualization of the splenic or portal vein was obtained.

Splenic punctures were performed through an intercostal space using an 18 gauge needle. Following splenic aspiration, approximately 25 ml. of 70 per cent Diodrast was injected through the same needle and serial radiographs were obtained at one second intervals using a Sanchez-Perez rapid cassette changer.

With this technique splenic vein obstructions and abnormalities of the portal circulation, such as otherwise undetectable gastroesophageal varices, were demonstrated. In addition, portal radicles in the liver were visualized.

This procedure is not without danger. Although no fatalities were encountered, one instance of splenic tear occurred. It is recommended, therefore, that splenography be employed only in those patients with an easily palpable, firm spleen in whom splenectomy is not contraindicated. Following splenography, patients should be observed closely for signs of intra-abdominal hemorrhage so that splenectomy may be performed if necessary.

In our experience the risk associated with splenography is slightly greater than that associated with splenic aspiration. It should, however, be employed only with full recognition of the possible danger, and only if facilities are available for dealing with complications if they should occur.

It is concluded that splenography may be employed routinely, with or without splenic aspiration, immediately prior to splenectomy. In such cases thrombocytopenia does not constitute a contraindication and the procedure is apparently without danger.

197. CORRELATION OF RELEVANT CLINICAL SIGNS WITH LEFT ATRIAL PRESSURE PULSE WAVES IN PATIENTS WITH MITRAL VALVE DISEASE

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At thoracotomy we have recorded electronically through a catheter the left atrial pressure pulse waves of 57 patients with healed rheumatic endocarditis. Fifty patients were found to have predominant mitral stenosis, 7 predominant mitral insufficiency. Previously we have described five serial groups of qualitative abnormalities in these waves: Group I, normal a-c-v-wave configuration at supernormal pressure levels; Group II, a small regurgitant wave interposed after the c-wave; Group III, loss of the trough between the c- and v-waves; Group IV, an undulating upstroke; Group V, a single, smooth wave.

Recent analysis of the tracings in relation to clinical observations on these patients has revealed the following pertinent facts: (1) Generally, patients with dominant mitral stenosis (44 of 50) fall in Groups I to III, while those with dominant insufficiency (6 of 7) fall in Groups III to V. (2) Twenty-six patients had apical systolic murmurs louder than Grade I (Scale I to VI). No Group I patient had the murmur. The proportion of patients having it increased progressively in Groups II to V. Surgeons felt regurgitant jets in 18 of 26 patients with the murmur. (3) Left atrial size tended to be larger in Groups IV and V, and agreement between roentgenologic prediction and surgical estimation of size was good. (4) A small, sharp wave occurring after descent of the v-wave appeared in 9 tracings. Eight of these patients had an opening snap of the mitral valve preoperatively, and the ninth had it postoperatively. (5) Heart rate definitely influences wave configuration: in one patient the preoperative occluded distal pulmonary artery tracing at a slow heart rate showed a Group I wave, whereas the operative left atrial tracing at a rapid heart rate showed a Group IV wave.

It is of collateral interest that aortic pulsation appears to influence the contour of left atrial pressure pulse waves. In some patients with simultaneous records we have found the left atrial v-wave beginning to descend synchronously with the descending limb of the aortic systolic pressure pulse wave before closure of the aortic valve, suggesting transmission of the aortic wave.

198. NUTRITIONAL MANAGEMENT OF CHILDREN WITH DIABETES MELLITUS

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The maintenance diet of the child with diabetes mellitus should be essentially the same as for normal children. We are presenting the caloric intake and growth curves for 71 juvenile diabetics, comparing the nutritional value of their diets with the 1953 recommended allowances of the National Research Council, and discussing briefly the nutritional management of children with diabetes mellitus.

The children observed have maintained a high degree of control of their disease by taking accurate doses of specific types of insulin in relationship to the intake of a nutritionally complete diet adjusted to compensate for variations in physical activity. The length of continuous observation on a single subject ranged from 3 to 18 years, with the median for boys being 7 years, 9 months, and for girls 7 years, 8 months. The data on caloric intake tabulated from the records of these children are unique in that the diets were quantitated or semiquantitated.

This serial information taken during childhood and adolescence has added to our understanding of the total energy requirements of boys and girls, of the caloric requirements per pound of body weight, the differences in requirements for sex and activity, and the need for a reduction in calories for girls shortly after maturation. Curves and tables derived from these data may be used as a general guide for caloric requirements for children and adolescents.

A comparison of the nutritive value of the diabetic diets with the 1953 National Research Council recommended allowances reveals that, even though caloric intakes in general are lower than those recommended, the diets provide a more liberal allowance of protein, calcium, vitamin A, and riboflavin.

Information is given concerning the advisability of individualizing the diet to meet the varying needs of growth, physical activity, and maturation pattern of each child.

199. SERUM CONCENTRATION OF VITAMIN B_{12} IN NORMAL AND NUTRITIONALLY DEFICIENT MONKEYS

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(INTRODUCED BY PAUL S. RHOADS, M.D.)

WITH THE TECHNICAL ASSISTANCE OF ELEANORE LABAW, M.S. (BY INVITATION), AND MARTHA LONG, M.S. (BY INVITATION)

In a study of nutritional factors in the hematopoiesis of the *Macaca rhesus* monkey vitamin B_{12} serum concentrations have been determined in normal and nutritionally deficient animals.

Vitamin B_{12} serum levels were determined by *Euglena gracilis* microbiologic method using techniques which have been described previously for human serum assay. The results of assay are expressed as "total" and "easily available" vitamin B_{12}. Seventeen young monkeys maintained on normal diets for several months exhibited a total vitamin B_{12} concentration of from 50 to 234 $\mu\mu$g per milliliter of serum with a mean value of 120 $\mu\mu$g. Thirteen of these animals were found to have "easily available" vitamin B_{12} varying from 33 to 132 $\mu\mu$g per milliliter with a mean value of 69 $\mu\mu$g per milliliter. Over one-half of the total serum vitamin B_{12} content was in "easily available" form in contrast to human serum in which the range of total concentration is somewhat higher, but in which only traces of the vitamin are in the "easily available" form.

Animals of synthetic vitamin-free diet supplemented with vitamin rations gradually developed low serum concentrations of vitamin B_{12} when receiving 1 or 2 μg of the vitamin, but returned to high normal serum concentrations on 10 μg per day. Adequate "binding capacity" of the serum was demonstrated by the presence of high serum concentrations 48 hours after intravenous administration of 5 μg of vitamin B_{12}.

Folic acid deficient monkeys receiving 2 μg of vitamin B_{12} daily maintained normal serum levels although the vitamin B_{12} serum concentrations

increased during the period of recovery from folic acid deficiency. In contrast, animals on ascorbic acid deficient diets developed low vitamin B_{12} serum concentrations which returned to high levels during the phase of induced recovery from scurvy. These data suggest that scurvy, unlike folic acid deficiency, markedly interferes with vitamin B_{12} absorption but that absorption is accelerated during the recovery phase from either scurvy or folic acid deficiency.

Simultaneous serum and fecal vitamin B_{12} assays indicated that substantial amounts of the vitamin are excreted in the presence of low as well as high serum concentrations, suggesting gastrointestinal synthesis of vitamin B_{12}, some of which is not absorbed.

200. THE ELECTROPHORETIC PATTERNS OF SERUM PROTEINS IN PURPURA HEMORRHAGICA HYPERGLOBULINEMICA (WALDENSTRÖM)

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In 1943-1944 Waldenström reported 4 patients with purpura of the relapsing type occurring chiefly on the legs, mainly after exercise, and not combined with any other signs of a hemorrhagic disorder. The purpuric lesions were of short duration but characteristic in that they left pigmented spots on the legs. The sedimentation rate was always elevated. The typical leg lesions and the absence of any other "acceptable diagnosis" made it easy to suspect the disease. The gamma globulin was increased, did not disappear, and the outcome has never been fatal. He called the disease "purpura hyperglobulinemica." Later, Waldenström reported 3 additional cases. Only 2 additional cases have been published with observations on the gamma globulin.

We have been fortunate in observing 11 patients who fulfill the criteria of Waldenström. Two were males. The age range was from 23 to 70 years. The duration of the disease was from 13 months to 15 years. The intravenous coagulation time, bleeding time, clot retraction, and prothrombin and platelet levels were all within normal limits.

The electrophoretic pattern (filter paper method) was abnormal in all subjects, in that the gamma globulin peak was elevated, at times to a tremendous degree. In one patient the gamma peak was higher than the albumin. The effect of ACTH on the electrophoretic pattern was observed in one patient. Large doses were given for 15 days with no change in the abnormal pattern although the height of the peak decreased slightly.

Two additional female patients, 33 and 37 years of age, respectively, fulfilled the criteria of Waldenström with the exception that the platelets were 92,000 and 85,000 per cubic millimeter, respectively (by the direct method). In both the gamma globulin was markedly elevated.

Therapeutic trials of ACTH and cortisone were completely disappointing in all patients with hyperglobulinemica.

Thus a total of 13 patients were observed with Waldenström's type of purpura. The electrophoretic pattern of the serum proteins was abnormal in that the gamma globulin was increased. ACTH and cortisone had no therapeutic effect.

201. A METABOLIC AND THERAPEUTIC STUDY OF VARIOUS COBALAMINS IN MULTIPLE SCLEROSIS

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The blood of patients with multiple sclerosis has been said to contain toxic levels of cyanide, the amount being about one-tenth fatal levels. Mushett and associates studied the antidotal effects of vitamin B_{12a} (hydroxocobalamin) in experimental cyanide poisoning and were able to both revive and protect their animals. These two observations logically caused us to study the metabolism and therapeutic value of cobalamins other than cyanocobalamin in patients with multiple sclerosis. All cobalamins have a marked affinity for CN^-, being converted instantaneously to the ordinary vitamin B_{12} (cyanocobalamin). The molecular weights are such that 1,000 μg of cobalamins can bind only 18 μg of HCN.

In the present study a mixture of sulfitocobalamin and hydroxocobalamin was given intramuscularly to 13 patients with multiple sclerosis. The daily dosage was 2,000 μg. In 3 subjects the urinary excretion of CN^-, cyanocobalamin, and cyanide-free cobalamins was determined. The method of Boxer and Richards was used in determining these substances and also in preparing the cyanide-free cobalamins. Base-line studies revealed the following average 24-hour total urinary excretion: CN^-, 2.0 μg; cyanocobalamin, 30.5 μg; and cyanide-free cobalamin, 131 μg. During the injection of 2,000 μg of cyanide-free cobalamins per day, the following results were obtained: CN^-, 3.6 μg; cyanocobalamin, 231 μg; and cyanide-free cobalamins, 714 μg.

The patients were treated from 2 to 8 months. One patient received treatment for 16 months. Inasmuch as exacerbations of the disease occurred during this period of treatment, it was concluded that cyanide-free cobalamins have no beneficial effect and remissions observed were spontaneous. One patient was sensitive to B_{12} and an explosive neurological involvement occurred. It was further concluded that no significant amounts of cyanide are present to convert appreciable amounts of cyanide-free cobalamins to cyanocobalamin.

202. OBSERVATIONS ON ABNORMAL CLOT RETRACTION AND FIBRINOLYSIS IN POLYCYTHEMIA VERA

SLOAN J. WILSON, M.D., AND HELEN E. HEATH, A.B. (BY INVITATION), KANSAS CITY, KAN.

An increased bleeding tendency occurs in some individuals with polycythemia vera. There is no one cause, and each patient must be studied as an individual. When polycythemia vera is in an active phase with an increase in the red cell mass and hematocrit, prothrombin and fibrinogen are decreased per unit volume of whole blood. Another phenomenon that has been observed by several investigators in the "falling out" or separation of the erythrocytes when clot retraction occurs has been interpreted as an increase in fibrinolytic activity.

The present study was undertaken to determine the following: (1) Is the separation of red cells and fibrin clot a fibrinolytic phenomenon? (2) Is the separation phenomenon affected by therapeutic control of polycythemia vera? (3) Are the fibrinogen levels decreased per volume of plasma? In each case the retraction of the clot and the "falling out" of the erythrocytes were studied. Fibrinolysis was quantitatively determined by collecting whole blood in siliconed glassware without anticoagulants, cooling immediately in ice

water, centrifuging at 5° C., and separating the plasma into different 1.0 ml. aliquots. Thrombin was added for thorough clotting and the fibrin quantitatively determined. Other aliquots were incubated under sterile conditions at 37° C. for 24 and 48 hours. The fibrin clot was removed and chemically analyzed. Thus the fibrin destroyed was actually measured in milligrams.

The blood of 14 patients with polycythemia vera was observed. In 9 instances increased "falling out" of the red cells from the retracted clot was observed even though most patients were under therapeutic control with P^{32} and phlebotomy. Moderately increased lysis of the fibrin clot was noticed in only one patient. The fibrinogen levels were normal.

The following conclusions were reached: (1) The separation of the red cells from the fibrin clot and fibrinolytic activity cannot be correlated. (2) The separation phenomenon may still remain in patients under therapeutic control. (3) Fibrinolytic activity was observed in only one instance. (4) Fibrinogen levels per unit volume of plasma are not decreased and do not cause the separation phenomenon.

203. DIRECT ARTERIAL STUDY OF THE BLOOD PRESSURE RESPONSE TO COLD IN NORMOTENSIVE SUBJECTS AND PATIENTS WITH ESSENTIAL HYPERTENSION BEFORE AND DURING TREATMENT WITH VARIOUS ANTIHYPERTENSIVE DRUGS

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The cold pressor test, with immersion of the hand in water at 4° C. for one minute, was performed three times at 15-minute intervals on 65 normotensive subjects and on 60 patients with essential hypertension both before and during treatment with antihypertensive drugs. The arterial pressure was recorded continuously from the radial artery. The recording system consisted of a strain-gauge manometer and a Sanborn multichannel recorder with a flat frequency response to 12 cycles per second. Statistically the cold pressor response during each of the three tests in the normotensive and hypertensive groups was very consistent for each individual. The mean results of the three tests, therefore, were used for further analysis.

The cold pressor response differed markedly among individuals in the control group. Thirty-six subjects had a maximal direct diastolic pressure of less than 85 mm. Hg with a mean blood pressure rise of 17.2 mm. systolic and 11.1 mm. diastolic. Ten additional subjects had a maximal direct diastolic pressure of less than 85 mm., but their mean cold pressor response was 27.0/20.2. These two groups were considered "normal reactors." Nineteen subjects had maximal diastolic pressure of more than 85 mm. and a mean cold pressor response of 31.4/21.7 mm. They were called "normal hyperreactors."

The mean cold pressor response of the hypertensive group was 37.9/25.8 mm. before treatment and 34.0/20.0 mm. during treatment. Eleven patients with essential hypertension received protoveratrine and reserpine; 5 received hydralazine and reserpine; and 13 had hydralazine, protoveratrine, and reserpine. These combinations did not affect the cold pressor response. In 11 patients receiving hexamethonium and reserpine, the cold pressor response was moderately depressed. The cold pressor response was markedly depressed in 4 patients receiving pentapyrrolidinium; in 4 treated with hexamethonium, hydralazine and reserpine; in 6 treated with hexamethonium and hydralazine. It seems, therefore, that the ganglionic blocking agents markedly depress the cold pressor response, whereas the other drugs used have no effect on the response.

204. A COMPARATIVE STUDY OF THE DISTRIBUTION OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCI AMONG DIFFERENT ENVIRONMENTAL GROUPS

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The alarming increase in antibiotic-resistant staphylococci isolated from patients in the University of Minnesota Hospitals prompted a comparative study of coagulase-positive strains isolated from different environmental groups. Cultures obtained during a period of six months in 1953-1954 included 68 strains from nasopharynges of 208 hospital personnel; 41 from the nasopharynges of 2 outpatients; and 96 from 66 patients having staphylococcal sepsis.

In vitro resistance was determined for each strain with the following antibiotics: penicillin, streptomycin, chlortetracycline, oxytetracycline, chloramphenicol, bacitracin, and erythromycin. Bacteriophage typing was performed on each strain with 34 phages.

Only 5.2 per cent of the strains from the hospitalized patients were sensitive to 1 or less units of penicillin, while 40 per cent were resistant to more than 1,000 units. Similar trends were shown for streptomycin and the tetracycline drugs. Corresponding results were obtained with isolates from hospital personnel. However, over 60 per cent of the isolates from outpatients were quite sensitive to penicillin and to the other antibiotics, and no highly resistant cultures were encountered. The outpatients came mostly from rural areas, and presented no history of having received an antibiotic recently. Strains of staphylococci from all three groups were sensitive to chloramphenicol, erythromycin, and bacitracin. At the time this study was made these antibiotics were not widely used in the hospital.

Bacteriophage typing was successful with almost 70 per cent of all strains. The majority of antibiotic-resistant strains fell into the Group III phage types, whereas the isolates from the outpatients were not similarly restricted to any single phage group.

These results emphasize that antibiotic-resistant staphylococci appear in a healthy population, and in patients when residing in an environment where large quantities of antibiotics are being used. Resistance to each antibiotic is roughly correlated with the quantities of antibiotic used. Bacteriophage typing confirms the finding that the appearance of resistant staphylococci is a property of certain strains, occurring most frequently among the Group III phage types.

205. THE PREVENTION OF ADRENAL ATROPHY DURING PROLONGED TREATMENT WITH LARGE DOSES OF ORAL HYDROCORTISONE

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(INTRODUCED BY GORDON B. MYERS, M.D.)

While small doses of oral hydrocortisone may be used safely for long periods, they are usually inadequate therapy for serious disorders. Daily administration of more than 40 or 60 mg. regularly induces severe adrenal unresponsiveness by suppressing endogenous corticotropin. Abrupt withdrawal may cause serious illness and, if undertaken during stress, may induce fatal adrenal insufficiency. That these serious liabilities do not result from hypo-

secretion of corticotropin is shown by their infrequency in even mild form after abrupt cessation of long-term corticotropin and by positive evidence that long-term corticotropin patients do increase corticoid output promptly during severe stress. This study explores the ability of weekly doses of long-acting corticotropin to prevent adrenal atrophy from developing during long-term oral hydrocortisone therapy. The patient's 24-hour eosinopenic response to a standard 100 unit dose of long-acting corticotropin was first established. Thereafter, 80 to 120 mg. of oral hydrocortisone was given on the first five days of each week, a test dose of long-acting corticotropin on the sixth day, and no medication except the usual evening dose of hydrocortisone on the seventh day. The 24-hour eosinopenic response to the weekly corticotropin dose was determined and its size adjusted to maintain an eosinopenic response equivalent to that before therapy. This was usually accomplished by a weekly dose of 150 units when 80 mg. of hydrocortisone was given daily, and 200 units when daily hydrocortisone was 120 mg.; adrenal responsiveness being maintained, respectively, above 67 per cent and above 50 per cent of pretreatment. In several patients, abrupt termination of this regimen produced no clinical or laboratory suggestion of hypocorticism. A simpler program for clinical routine use combines six days of oral cortisone or hydrocortisone and one of long-acting corticotropin; steroid dosage is adjusted according to symptoms, corticotropin dosage to produce an eosinophil count below 10 per cent of basal eight hours after each weekly dose. The essence of this approach is that it combines prevention of adrenal atrophy with a simple weekly demonstration of its nonexistence. Preliminary discussion will be given of a technique of corticoid therapy which employs weekly or semiweekly injection of a long-acting corticotropin-hydrocortisone acetate mixture and depends for its safety on similar principles.

206. DIAGNOSTIC VALUE AND THEORETICAL SIGNIFICANCE OF THE UROPEPSIN:CREATININE RATIO IN CASUAL UNTIMED URINES

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(Introduced by Gordon B. Myers, M.D.)

Uropepsin determinations are rapid and simple by the recent homogenized milk or buffered casein techniques. This estimation probably has not received wide acceptance because diurnal variations in uropepsin excretion seemed to make 24-hour urine samples necessary. We find no significant difference between the uropepsin:creatinine ratio of casual day and night urines, presumably because known diurnal variation of creatinine excretion matches that of uropepsin. Subsequent observations suggest that the uropepsin:creatinine ratio of casual untimed urine samples yields information similar to that obtained from 24-hour urine uropepsin. Both are increased by hydrocortisone but not by corticosterone. A portion of the data on the effect of smoking and of the ulcer constitution are summarized in the accompanying table.

UROPEPSIN: CREATININE	NORMAL ADULTS NONSMOKERS	NORMAL ADULTS SMOKERS	NORMAL ADULTS ALL SUBJECTS	DUODENAL ULCER
Below .100	1	0	1	0
.100-.299	29	4	33	0
.300-.499	8	11	19	1
.500-.699	0	1	1	5
Over .700	0	0	0	8

From these preliminary data, examination of a single casual urine in suspected duodenal ulcer will be diagnostic in a majority of patients, suggestive in most others, but rarely misleading. Since little more than one hour is required for estimation of the uropepsin:creatinine ratio, it would appear to be of value in routine gastroenterological problems, in identifying ulcer-prone subjects prior to corticoid therapy, and in differential diagnosis of gastrointestinal hemorrhage.

207. ANTIBODY RESPONSE OF PERSONS WHO HAVE RECEIVED RABIES VACCINE

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The purpose of this study was to qualify the nature of antibody response to rabies vaccine. Eighty-one patients, who received 14 daily injections of vaccine, were studied. Serum was obtained before treatment on the seventh and fourteenth days, and from 4 to 20 weeks later. Complement-fixing antibody titers were determined using a twofold tube dilution. The antigens used were: lyophilized benzene extracted adult human brain (HB); identically prepared fetal human brain (FB); irradiated virus infected rabbit brain (VAC); and uninfected rabbit brain, identically prepared (RB).

Among 81 patients, 6 had demonstrable titers averaging 2.7 tubes against HB and 10 had an average titer of 3.1 tubes against vaccine by the eighth day. On day 14, HB and VAC titers were present in 79 and 91 per cent, respectively. From 4 to 20 weeks later, titers against VAC were present in 85 per cent and against HB in 58 per cent. A 32 per cent average titer fall in 8.2 weeks occurred in 76 sera positive to VAC on day 14, or on final determination. A 53 per cent fall occurred in the 66 sera positive to HB.

The antibody response to VAC and RB was compared in 31 patients. The titer against VAC at 14 days was 26 per cent greater than the titer to RB. Because of this discrepancy, absorption studies were done in 23 patients. Sera were absorbed with VAC and with RB. The two groups of absorbed sera and a control of unabsorbed sera were each titered against the two antigens. Both VAC and RB absorbed sera yielded significantly higher residual titers to VAC than to RB, with chi square values of 16 and 11.2, respectively. Residual titer to VAC was approximately the same after VAC or RB absorption. The results indicate that the antibody response to vaccine is composed of one which successfully absorbs brain antigen and a residual which may represent virus activity.

Of the 37 sera positive to HB, only 3, with titers no greater than 4+ at a 1:2 dilution, were positive to FB. Whereas HB successfully absorbed anti-brain antibodies, FB failed to do so.

208. SERUM GLUTAMIC OXALACETIC TRANSAMINASE AS AN INDEX OF LIVER DESTRUCTION AND DISEASE

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(INTRODUCED BY RICHARD H. LYONS, M.D.)

Glutamic oxalacetic transaminase has been found in all human sera studied. This enzyme is present in high concentration in liver as well as other tissues such as heart, kidney, muscle, and brain. Variations in serum transaminase in more than 100 patients with various types of liver diseases will be presented and correlated with other liver function tests.

In human as well as experimental animals, destruction of liver tissue by carbon tetrachloride results in elevation of serum transaminase by 50 to 1,000 times normal. The levels remain high for variable periods and fall with or before clinical improvement. Similar findings have been noted in hepatitis, invasion of liver tissue by neoplasm, and to a lesser degree in cholangitis. Cirrhotics have variable levels which appear to rise during activation of their disease. The height of transaminase activity and the pattern of serial determinations appear to be worthy of further study in the evaluation of liver disease.

209. IDIOPATHIC AND ACQUIRED SYMPTOMATIC AGAMMAGLOBULINEMIA

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(INTRODUCED BY GORDON B. MYERS)

Agammaglobulinemia, now recognized in both children and adults, is a pathophysiologic syndrome which may be due to congenital or acquired factors. The congenital types are simply diagnosed and amenable to replacement therapy. Four affected men have shown unusually high Howe and true A:G ratios; absent gamma-globulin and low beta-2-globulin; extreme susceptibility to infection; total absence of circulating and fixed antibodies, both inherited and acquired; no antibody response to antigenic challenge; negative flocculation tests despite proved liver damage, high fever, and elevated ESR. Three cases had familial lymphopenic agammaglobulinemia, presumably a sex-linked recessive disorder with clinical manifestations only in homozygous males and no manifestations in heterozygotes. Accompanying deficiency of tissue lympocytes, absent lymph node germinal centers, and peripheral blood lymphopenia suggest a primary deficiency of tissue lymphocytes. Familial nonlymphopenic dysglobulinemia has been observed in one man, whose agammaglobulinemia was not associated with abnormal lymph nodes or blood lymphocytes; relatives of both sexes have shown hypergammaglobulinemia. It may be a disturbance in globulin synthesis so controlled by multiple alleles that one abnormal gene deranges immunoglobulin synthesis and produces hypergammaglobulinemia, while two abnormal genes render immunoglobulin synthesis impossible.

Acquired agammaglobulinemia has been noted in a patient with beta-myeloma. Impaired resistance to infection was a prominent clinical feature, but the presence of isohemagglutinins indicated that the immunoglobulin-synthesizing mechanism was not completely destroyed. Autopsy findings will be described.

The metabolism of gamma-globulin in these disorders and their differentiation from other hypogammaglobulinemic illnesses will be discussed.

210. RENAL DEFECT ASSOCIATED WITH SICKLE CELL TRAIT AND SICKLE CELL DISEASE

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An evaluation of renal function in patients with sickle cell anemia and sickle cell trait was undertaken. Nineteen patients, ranging in age from 17 to 49 years, have been studied. Tests included urine analyses, Addis counts, 18-hour urine concentration tests, urea and creatinine clearances, and phenol-

sulfonephthalein excretion. Hickey-Hare tests were performed in selected individuals. In addition to the usual hematological and sickling tests, quantitative osmotic fragility test, fetal hemoglobin estimation, and in certain instances hemoglobin electrophoresis, and ABO, MN, and RhHr genotyping were also performed.

In every case there was a striking limitation in the urinary concentration function. Specific gravities after 18-hour fluid deprivation ranged from 1.007 to 1.018, and were not increased when repeated after the intramuscular injection of 5 units Pitressin. Urine dilution to specific gravities of 1.001 or 1.002 was achieved through hydration, while maximum concentrations of 1.011 to 1.016 followed intravenous injection of hypertonic saline and 0.1 unit Pitressin. Except for a few isolated instances, results of other renal function tests were within normal limits.

These studies indicate that an inherent defect in urine concentration function is exhibited by individuals with either sickle cell trait or sickle cell anemia. That this phenomenon has a genetic basis is further supported by studies of three generations of a family. The mother and father, both with sickle cell trait, and two sons with sickle cell anemia, manifest the renal defect. The paternal grandfather and the third son have no sickle hemoglobin and are able to concentrate urine normally. It is possible that low hemoglobin values may influence the concentration function, but it is not the cause since the deficiency was present in two trait individuals who had hemoglobin values of 14.2 Gm. and 13 Gm., respectively. Furthermore, patients with thalassemia and other equivalent anemias of nonsickle origin have so far not shown the urine concentration defect.

211. OBSERVATIONS ON THE SOURCE OF ELEVATED BLOOD "AMMONIA" LEVELS IN HEPATIC DISEASE

H. J. Zimmerman, M.D., R. J. Korn, M.D. (By Invitation), and H. G. Weinstein, M.D. (By Invitation), Chicago, Ill.

Elevation of the blood "ammonia" levels has been correlated with impending and established hepatic coma. Reports in the literature have suggested that the ammonia is released from foods by intestinal bacteria. Shunting of portal blood past the liver, rather an impaired parenchymal function, has been considered responsible for the elevation observed in severe hepatic disease. Studies performed included observations of fasting ammonia levels in patients without hepatic disease, with hepatitis, cirrhosis, and in those with hepatic coma. The mean blood ammonia level was slightly higher in patients with cirrhosis than in those without hepatic disease, and considerable overlapping values. Patients with hepatic coma frequently, but not invariably, had elevated levels. Although patients with hepatitis usually showed levels in the normal range, in a few with severe disease the levels were distinctly elevated, returning to normal with convalescence. Tolerance to oral and intravenous ammonium chloride and to oral gelatin was also studied. A somewhat lesser tolerance for oral and intravenous ammonium chloride as shown by more prolonged blood level elevations was seen in patients with cirrhosis than in those without hepatic disease. The oral administration of gelatin was followed by a rise in blood NH_3 levels. Prior administration of Aureomycin prevented this rise.

These studies confirm the gastrointestinal source of much of the blood ammonia, and are consistent with the impaired conversion of ammonia to urea by the patient with liver disease. They suggest that shunting of portal blood past the liver is only one factor, and that impaired parenchymal hepatic function also plays a role.

The Journal of Laboratory and Clinical Medicine

The Official Publication of the Central Society for Clinical Research

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950 East 59th Street
Chicago 37, Illinois

PUBLISHED BY THE C. V. MOSBY COMPANY, 3207 WASHINGTON BLVD., ST. LOUIS 3, U. S. A.

Published monthly. Subscriptions may begin at any time.

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